Universitat Autònoma de Barcelona

Factores Genéticos asociados a la Degeneración Lobar Frontotemporal

Análisis de susceptibilidad genética y correlación fenotipo-genotipo

Doctoranda
Mª Isabel Hernández Ruiz

Directores
Agustín Ruiz Laza
Mercé Boada Rovira
Rafael Blesa Gonzalez

Tutor
José Álvarez Sabin

PROGRAMA DE DOCTORADO EN MEDICINA
DEPARTAMENTO DE MEDICINA
UNIVERSITAT AUTÒNOMA DE BARCELONA
2014

DIRECTORES DE TESIS

Dr. AGUSTÍN RUIZ LAZA, Doctor en Medicina por la Universidad de Sevilla.
Dra. MERCÉ BOADA ROVIRA, Doctora en Medicina por la Universidad de Barcelona.
Dr. RAFAEL BLESA GONZALEZ, Doctor en Medicina por la Universidad de Barcelona.

TUTOR DE TESIS

Dr. JOSÉ ÁLVAREZ SABIN, Doctor en Medicina por la Universidad de Barcelona. Profesor Titular de Neurología de la Universitat Autònoma de Barcelona.

CERTIFICAN

Que la tesis titulada ***"Factores Genéticos asociados a la Degeneración Lobar Frontotemporal. Análisis de susceptibilidad genética y correlación fenotipo-genotipo"***, presentada por Mª Isabel Hernández Ruiz, se ha realizado bajo nuestra supervisión y consideramos que reúne los requisitos necesarios para ser defendida ante el Tribunal correspondiente para optar al grado de Doctor en Medicina por la Universitat Autònoma de Barcelona.

Agustín Ruiz Laza

Cap de Recerca
Fundació ACE (ICNA)
Barcelona

Mercè Boada Rovira

Directora Mèdica
Fundació ACE (ICNA)
Barcelona

Rafael Blesa González

Cap de Servei Neurología
Hospital de la Santa Creu i Sant Pau
Barcelona

José Álvarez Sabin

Cap de Servei de Neurología
Hospital de la Vall d'Hebrón
Barcelona
Professor Titular de Neurología
Universitat Autònoma de Barcelona

Barcelona a 15 de Septiembre de 2014

"Enseñarás a volar,
pero no volarán tu vuelo.

Enseñarás a soñar,
pero no soñarán tu sueño.

Enseñarás a vivir,
pero no vivirán tu vida.

Sin embargo...
en cada vuelo,
en cada vida,
en cada sueño,
perdurará siempre la huella
del camino enseñado"

Madre Teresa De Calcuta

A mi padre,
que supo transmitirme el valor del respeto y la generosidad

AGRADECIMIENTOS

Hace 20 años aposté, como neuróloga, que iba a dedicar el resto de mis años de actividad profesional al mundo de las demencias. No me he equivocado. La demencia es para mí una de las enfermedades más dura y compleja que existe. El tiempo dedicado a los pacientes, la confianza que han depositado en mí, las frustraciones sufridas por no poder ofrecerles más y su pérdida final me han demostrado que lo más importante en la vida son las personas, sus emociones y sus sentimientos. Haberme dejado compartir con ellos esta experiencia ha sido todo un honor.

Es por eso que mi primer agradecimiento va dedicado a ellos, por los años que me han dedicado y por permitirme acompañarlos en su largo "viaje a ninguna parte". Sin ellos este trabajo no hubiera sido posible. A sus familiares y cuidadores, por transmitirme todo aquello que "ellos" no podían apreciar, sentir o expresar. Por su constante labor de cuidado y amor hacia alguien que algún día fue y que ya no es.

Reconocer que fueron Lluis y Mercè los primeros en creer y apostar por mí, dejándome acompañarles en su sueño desde el principio y manteniéndome a su lado, como profesional y amiga, hasta la realidad de hoy. También por su insistencia y ayuda en la elaboración de este trabajo. Gracias a los dos.

A Pilar, Montse, Anna y Ana, Maiteé, América y Marina, a Charo y a todos los que han compartido conmigo la realidad de Fundació ACE todo este tiempo. Su compañía, risas y alegrías, dedicación, apoyo y trabajo han contribuido a que el mío haya sido más agradable todos estos años. Un especial recuerdo para "nuestra Rosa". Donde quiera que estés siempre te llevaré conmigo.

A "Agus" por su entrega y dedicación como "Director Becario" y por introducirme en el mundo de la genética donde, a pesar de sus enseñanzas, sigo siendo una aprendiz. Sin él este trabajo no hubiera visto la luz.

A mis colegas de profesión, porque todos me han enseñado algo.

A José Mª por haber estado siempre a mi lado acompañándome en mi vida profesional, siempre en segundo plano, pero apoyándome y haciéndome avanzar en los momentos difíciles.

A Victoria y Ana que siempre han sido mi prioridad, mi razón de ser y mi continua preocupación como madre.

Finalmente a Nieves, mi madre, porque su pérdida precoz me motiva en la lucha contra estas enfermedades que hoy son aún incurables.

ÍNDICE

I. Glosario de abreviaturas

APP: Afasia Progresiva Primaria

APPnf: Afasia Progresiva Primaria no fluente

APPvs: Afasia Progresiva Primaria variante semántica

APPvl: Afasia Progresiva primaria variante logopénica

aDLFT-U: DLFT atípica con inclusiones de ubiquitina,

BIBD: Enfermedad por cuerpos de inclusión basofílicos

CHMP2B: Charged multivesicular body protein

C9orf72: Chromosome 9 open reading frame 72

DLFT: Degeneración Lobar Frontotemporal

DFT: Demencia Frontotemporal

DFTvc: Demencia Frontotemporal variante de conducta

DFTP-17: Demencia Frontotemporal con Parkinsonismo asociada al cromosoma 17

DFT-ELA: Demencia Frontotemporal con Esclerosis Lateral Amiotrófica

DFLT-U: Degeneración Lobar Frontotemporal por depósitos que se tiñen con Ubiquitina

DLFT-TAU: Degeneración Lobar Frontotemporal por depósitos de proteína Tau

DFLT-TDP: Degeneración Lobar Frontotemporal por depósitos de proteína TDP-43

DLFT-FUS: Degeneración Lobar Frontotemporal por depósitos de proteína FUS

DLFT-UPS: Degeneración Lobar Frontotemporal con inmuno-histoquímica contra proteínas del sistema ubiquitina-proteasoma

DLFT-ni: Degeneración Lobar Frontotemporal sin inclusiones

DGA: Demencia por Granos Argirófilos

EA: Enfermedad de Alzheimer

EMN: Enfermedad de Motoneurona

ELA: Esclerosis Lateral Amiotrófica

FUS: (gen) Fused in sarcoma

FUS: Tumor associated protein fused in sarcoma

GRN: (gen) Progranulina

MAPT: Microtubule associated protein Tau gen

NIFID: Enfermedad por inclusión de filamentos neuronales intermedios

PSP: Parálisis Supranuclear Progresiva

P62: Proteína de unión a ubiquitina

SCB: Síndrome Cortico Basal

SQSTM1: (gen) sequestrosoma

TARDBP: (gen) TAR DNA Binding Protein

Tau: Proteína Tau asociada a microtúbulos

TDP-43: Transactive response DNA binding protein of 43 kD

TMEM106B: Transmembrane protein 106B

TREM2: (gen) Triggering receptor expressed on myeloid cells 2

UBQLN2: (gen) Ubicuitin 2

VCP: (gen) Valosin-containing protein

II. Índice de Tablas y Figuras

TABLAS

FIGURAS

ABSTRACT

La Degeneración Lobar Frontotemporal es un grupo heterogéneo de enfermedades, la segunda causa más frecuente de demencia en edad presenil y la que presenta el mayor número de casos hereditarios. Se caracteriza por una gran variabilidad clínica, genética e histopatológica. Las personas afectas pueden presentar síntomas que abarcan desde los trastornos de conducta hasta las diferentes alteraciones del lenguaje, con o sin enfermedad de motoneurona o parkinsonismo asociado. La atrofia en los lóbulos frontales y temporales es el hallazgo radiológico más relevante. En los últimos 10 años el conocimiento de esta entidad clínica ha presentado remarcables cambios a nivel genético e histopatológico, que han servido para establecer criterios clínicos más consistentes. Hasta el momento han sido descritos diez genes asociados a DLFT y cuatro diferentes proteínas de agregación han sido detectadas en los cerebros afectados. Este trabajo aporta la experiencia clínica de más de 15 años en pacientes con DLFT y el trabajo de colaboración con diferentes grupos de investigación en genética de enfermedades neurodegenerativas.

Frontotemporal Lobar Degeneration is a heterogeneous group of disorders, the second most frequent cause of early dementia and the one with the highest number of inherited cases. It is characterized by considerable variability in clinical, genetic and histopathology features. Patients may present symptoms ranging from behavioral disturbances to different language disorders, with or without motor neuron disorders or associated Parkinsonism. Atrophy in frontal and temporal lobes is the most relevant radiological finding. In the last 10 years, the knowledge of this clinical entity has undergone remarkable changes both genetically and histopathologically, which have served to establish more consistent clinical criteria. Until now 10 genes causative of dementia have been described and up to four different proteins causative of atrophy have been detected in aggregates. This work provides the clinical experience of more than 15 years with DLFT patients and the collaborative work with different Genetic Research Groups in Neurogenerative Disorders.

III.- Introducción
Revisión y puesta al día

La Degeneración Lobar Frontotemporal (DLFT) se caracteriza por una pérdida selectiva, específica y progresiva de neuronas localizadas preferentemente en las regiones frontales y temporales del cerebro humano. Una misma población neuronal puede ser objeto diana de diferentes patologías (proteotipo), por lo que la sintomatología de presentación clínica (fenotipo) va a variar dependiendo del área afectada.

Representan un grupo de enfermedades con unas bases clínicas, moleculares y genéticas heterogéneas, donde convergen a menudo los mecanismos neurodegenerativos con el fenotipo clínico de presentación. El espectro clínico varía desde la sintomatología de conducta hasta los síndromes afásicos progresivos, parkinsonismo plus y/o enfermedad de motoneurona, asociándose a menudo varios síndromes en un mismo sujeto a lo largo de la evolución clínica (Thelen et al. 2014). Se caracterizan fundamentalmente por cambios progresivos de conducta, disfunción ejecutiva y dificultades de lenguaje.

La historia natural de la DLFT va a depender de múltiples factores, tanto intrínsecos (biológicos, genéticos) como extrínsecos (ambientales y sociales). Los factores intrínsecos, implícitamente impuestos, no son controlables por el clínico y van a definir el fenotipo de presentación. No es así con los extrínsecos, que son los que marcarán la variabilidad en la evolución de un fenotipo determinado. Es por ello que estas enfermedades neurodegenerativas han de ser valoradas en su contexto global, clínico y social, para poder ofrecer, tanto al paciente como a la familia, una orientación adecuada.

Hasta 1994, en que los grupos de investigación de Lund y Manchester proponen los criterios clínicos para el diagnóstico de DLFT (Statement 1994), estos pacientes eran comúnmente diagnosticados de Enfermedad de Alzheimer (EA). Es en el año 1998 (Neary et al. 1998) que son publicados los criterios diagnósticos para este tipo de enfermedad neurodegenerativa, que la diferencia clínicamente y de forma clara de la EA. Por sus peculiares características clínicas, es una patología que en ocasiones genera dudas y orientaciones diagnósticas equivocadas, dando lugar a tratamientos y recursos sociales alejados de las necesidades del caso.

La DLFT es la segunda causa más común de demencia en individuos menores de 65 años, representando el 5-15% de todas las demencias en este grupo de edad (Bird et al. 2003) (después de EA), y la tercera causa en mayores de 65 después de la demencia por cuerpos de Lewy (Arvanitakis 2010).

La prevalencia de la DLFT varía según las series analizadas: desde 2.7/100.000 y 9.4/100.000 en el grupo de 60-69 en la serie de Netherlands (Rosso et al. 2003) hasta 15.1/100.000 en la serie de Cambridge (Ratnavalli et al. 2002). La prevalencia más baja se informa en la serie de Japón, con un 2.0/100.000 (Ikejima et al. 2009) y la más alta en Italia, con un 31/100.000 (Gilberti et al. 2012). Está considerada una demencia presenil y supone el 20-25 % de los todos los casos de demencias que se presentan alrededor de los 65 años, aunque en la serie de Sweden (Gislason et al. 2003) se encontró una prevalencia del 3% en el grupo de edad de 85 años. En una serie anatomopatológica de Newcastle General Hospital (Baborie et al. 2012), el 3.4 % de los pacientes seniles autopsiados presentaban criterios de DLFT, con una edad media de 73.5 [65-92] circunstancia que hace pensar que posiblemente esta infradiagnosticada en este grupo de edad.

En los últimos 15 años, el espectro de la DLFT ha cambiado notablemente, tanto a nivel clínico como en el conocimiento de la genética asociada y las proteínas de depósito implicadas. No es así en el área de tratamiento, huérfanas aún de posibles terapias.

Para mejor comprensión de los términos, DLFT hace referencia a la enfermedad y su diagnóstico anatomopatológico en todas sus variantes y DFT al síndrome clínico de cualquier presentación fenotípica.

1. FENOTIPOS DFT. CLASIFICACIÓN Y CARACTERÍSTICAS CLÍNICAS.

Las DFT se clasifican, de acuerdo a la característica clínica principal observada en el paciente, como: demencia frontal variante de conducta (DFTvc), afasia progresiva primaria (APP): variante semántica o demencia semántica (DS), afasia progresiva no fluente (APNF), síndrome cortico basal (SCB), síndrome de parálisis supranuclear progresiva (SPSP) y DFT con enfermedad de motoneurona (DFT-EMN).

a. Demencia Frontotemporal variante de conducta (DFTvc)

La DFTvc comprende más de la mitad de los casos de DLFT y es su fenotipo hereditario más común. Su debut suele presentarse antes de los 65 años, con una media a los 58 años (K. A. Josephs et al. 2011). Se caracteriza por cambios precoces en la personalidad y la conducta, tales como la desinhibición, a menudo coexistiendo a lo largo de la evolución con apatía, impulsividad, falta de empatía, conductas estereotipadas y pérdida de competencia y conducta social. Las funciones ejecutivas se encuentran alteradas precozmente estando la memoria y las funciones visuo-perceptivas bien preservadas, tal como revelan los test cognitivos (Sieben et al. 2012). Un grado variable de alteración de lenguaje está también presente y la hiperoralidad y los cambios en los hábitos alimentarios son a menudo comunes, dando lugar a un notable aumento de peso.

De acuerdo con los criterios de "International Behavioral Variant FTD Consortium" (Rascovsky et al. 2011) se clasifica en: DFTvc posible, sólo por criterios clínicos y requiriendo la presencia de tres de seis signos de trastornos de conducta y cognición: desinhibición , apatía/inercia, pérdida de empatía, conducta perseverativa e impulsiva, hiperoralidad y perfil neuropsicológico disejecutivo; DFTvc probable cuando se observa declive funcional y la neuroimagen soporta los criterios de posible y DFTvc "definitiva" cuando se dispone de confirmación neuropatológica o evidencia de mutación genética conocida.

Teniendo en cuenta sólo la neuroimagen, cuatro subtipos han sido identificados dependiendo de la pérdida de sustancia gris relativa observada: frontal dominante, frontotemporal, fronto-temporo-parietal y temporal dominante (Whitwell et al. 2009). El subtipo frontal dominante engloba los lóbulos frontales y la ínsula anterior. El subtipo frontotemporal muestra afectación de los lóbulos frontales, la ínsula anterior, el caudado y el putamen y lóbulo temporal anterior derechos. El subtipo fronto-tempo-

ro-parietal muestra mayor pérdida de materia gris en comparación con el subtipo dominante temporal, caracterizado por la implicación del lóbulo temporal, particularmente derecho, medial e inferior.

b. Afasia Progresiva Primaria (APP)

Se debe aplicar el término Afasia Progresiva Primaria a aquella alteración del habla o el lenguaje que se presenta, durante un periodo de al menos dos años, como única queja cognitiva (Mesulam 1982). Pacientes con DFT y dificultades lingüísticas habían sido diagnosticados, a lo largo de los años, en dos categorías: afasia progresiva no fluente (APNF) y demencia semántica (DS). Sin embargo hay pacientes con APP cuyas características clínicas no cumplen ninguno de esos criterios. Es en 2011 cuando se publican las nuevas recomendaciones para la sub-clasificación de las APP, describiendo tres subtipos diferenciados: (ML Gorno-Tempini et al. 2011) afasia progresiva primaria no fluente (APPnf), afasia progresiva primaria variante semántica (APPvs) y afasia progresiva primaria variante logopénica (APPvl).

► APPnf es la segunda presentación clínica más prevalente dentro de las DFT, representando alrededor del 25% (Johnson et al. 2005a) y caracterizada por simplificación gramatical, esfuerzo y vacilación en el habla con errores en la emisión de sonidos y la producción del lenguaje. La comprensión lingüística se encuentra relativamente preservada así como el resto de las funciones cognitivas (Grossman 2012). Es frecuente que la afasia se acompañe de apraxia del habla y orolingual (K. A. Josephs et al. 2011). Durante la evolución el lenguaje se vuelve telegráfico, tanto en la expresión oral como en la escritura, seguido de un gradual deterioro de la comprensión de frases y finalmente mutismo y demencia (Turner et al. 1996). La apatía es el cambio de conducta asociado más común. La neuroimagen muestra anormalidades en la región fronto-insular posterior izquierda, giro frontal inferior, ínsula, áreas premotoras y motoras suplementarias, siendo necesarios estos hallazgos para realizar el diagnóstico de APPnf probable (M L Gorno-Tempini et al. 2011). A lo largo de la progresión clínica pueden aparecer signos extrapiramidales tipo SPS o SCB, dando lugar a cambios en el fenotipo y por lo tanto en el diagnóstico clínico.

► La APPvs se caracteriza por la pérdida del significado de las palabras (Hodges and Patterson 2007). Basándose en los criterios establecidos, los déficits de comprensión para palabras simples y la nominación por confrontación visual

son los signos principales y esenciales para el diagnóstico (M L Gorno-Tempini et al. 2011), aunque el habla es fluida y la sintaxis correcta. Las alteraciones de conducta también están presentes, como la falta de empatía y la inflexibilidad mental. Los déficits de reconocimiento de personas son especialmente presentes cuando el lóbulo temporal derecho es el afectado. La neuroimagen muestra una atrofia significativa de los lóbulos temporales mediales y laterales, aunque más llamativa en el lado izquierdo (Mummery et al. 2000).

► La APPvl se asocia, sobre todo, al diagnóstico neuropatológico de enfermedad de EA (Rabinovici et al. 2008) y no se considera parte del grupo de las DFT. La importante dificultad en encontrar las palabras (en el lenguaje espontáneo y en las tareas de confrontación verbal) y la alteración en la repetición de frases y oraciones, son los signos claves de esta variante afásica. Son necesarias y apoyan el diagnóstico de APPvl alteraciones radiológicas en las áreas temporo-parietal izquierda, temporal posterior y giros angular y supramarginal (ML Gorno-Tempini et al. 2004).

La APP se clasifica como "posible" basándose en las características clínicas. Se clasifica como "probable" cuando los hallazgos clínicos están apoyados por las técnicas de neuroimagen y sólo después del análisis posmortem o teniendo evidencia de la existencia mutación genética, la APP es clasificada como "definitiva" (ML Gorno-Tempini et al. 2011).

c. Síndromes asociados a la DFT. Síndrome Córticobasal (CBS) y Síndrome de Parálisis Supranuclear Progresiva (SPSP)

Ambos síndromes han sido descritos originalmente como trastornos del movimiento, parkinsonismos atípicos o "Parkinson Plus", pero muestran una asociación significativa con la DLFT desde el punto de vista clínico, genético y anatomopatológico (A Kertesz et al. 2000).

► El SCB presenta una prevalencia menor de 1/100.000 habitantes y se caracteriza por síntomas extrapiramidales de evolución progresiva, de tipo rígido y asimétrico y con distonía asociada. La pérdida sensorial cortical, síndrome del miembro ajeno, heminegligencia y mioclonias están presentes. Frecuentemente se presenta combinado con APPnf y en las etapas avanzadas de la enfermedad, con alteraciones de conducta (A Kertesz et al. 1994); y por el contrario, los pacientes que inicialmente presentaron DFTvc o APPnf, pueden con el tiempo

desarrollar los trastornos del movimiento característicos del SCB (I Ferrer et al. 2003) o SPSP.

► La PSP presenta una prevalencia de 3.1/100.000 habitantes y es un trastorno neurológico primario que se presenta con inestabilidad postural, parkinsonismo de predominio axial con retropulsión, enlentecimiento motor y parálisis de la mirada vertical. Disartria, disfagia y signos pseudobulbares son signos frecuentemente asociados (Litvan et al. 1996). La disfunción cognitiva se presenta por alteración de los circuitos frontosubcorticales, provocando disfunción ejecutiva, enlentecimiento psicomotor y alteración de la memoria de trabajo (Grafman, Litvan, and Stark 1995). El espectro de SPSP no sólo incluye la clásica PSP tipo Richardson sino también la PSP-parkinsonismo, que se presenta de forma más asimétrica, asemejándose a la enfermedad de Parkinson y al síndrome de acinesia pura, caracterizado por bloqueo de la marcha y falta de fluidez del habla como características más prominentes.

Cuatro formas de presentación fenotípica han sido aprobados recientemente por consenso para hablar de criterios clínicos de degeneración cortico basal (DCB) (Armstrong et al. 2013): síndrome cortico basal clásico (SCB), síndrome frontal con trastorno conductual y alteración espacial (SFC), variante no fluente agramática de afasia progresiva primaria (vnfaAPP) y síndrome de parálisis supranuclear progresiva (SPSP). Sin embargo, estos criterios precisan de futuras validaciones.

En el caso del SPSP y de cara a mejorar la precisión diagnóstica, el grupo de expertos del National Institute for Neurological Disorders and Stroke (NINDS) han publicado los criterios de NINDS-SPSP (Respondek et al. 2013), donde la combinación de criterios posibles y probables proporciona una sensibilidad más alta en la atención clínica rutinaria. Sin embargo los autores sugieren que los criterios de probable son preferibles para el reclutamiento de pacientes en los ensayos clínicos, donde el diagnostico específico y precoz es importante.

d. Síndromes asociados: Enfermedad de Motoneurona (EMN)

La EMN comprende un grupo de enfermedades con pérdida progresiva de neuronas motoras: Esclerosis Lateral Amiotrófica (ELA), Parálisis Bulbar Progresiva (PBP) y Esclerosis Lateral Primaria (ELP). Clínicamente, la EMN se manifiesta con debilidad progresiva, pérdida de masa muscular y espasticidad, produciendo la muerte por insuficiencia respiratoria a los tres años de media, después de su inicio, en el 50% de los pacientes.

La Esclerosis Lateral Amiotrófica (ELA) es la forma más común de presentación y se caracteriza, patológicamente, por la pérdida progresiva de neuronas motoras superiores en la capa 5 de la corteza y neuronas motoras inferiores en los núcleos motores del tronco cerebral y del asta anterior de la médula espinal. La asociación entre la demencia y la ELA se observó hacia finales del siglo XIX y sucesivamente ha sido publicada por muchos investigadores.

Los casos familiares y esporádicos de ELA pueden tener disfunción frontal, cambios de personalidad, conducta, planificación, organización y disfunción de lenguaje. Los síntomas de ELA pueden preceder, presentarse simultáneamente o seguir a los signos y síntomas de DFT, aunque los hallazgos más comunes son encontrar cambios cognitivos seguidos de la debilidad muscular (Achi and Rudnicki 2012). En el 25% de los pacientes de ELA, el fenotipo clínico de DFT más comúnmente asociado es la DFTvc y ocasionalmente la APP.

Un consenso clínico de diagnóstico para DFT y EMN, consistente en cuatro ejes, fue propuesto por un grupo de trabajo internacional en 2007 (Strong et al. 2009).

Eje I: diagnóstico clínico de EMN; Eje II: disfunción cognitiva y conductual; Eje III: manifestaciones no motoras adicionales; Eje IV: identificación de la presencia de modificadores de la enfermedad. Tres formas clínicas diferentes pueden ser identificadas de acuerdo con el consenso: ELA con alteración de conducta, ELA con alteración cognitiva y ELA con demencia comórbida (otras no DFT).

e. Demencia Frontotemporal con Parkinsonismo (DFTP-17)

La DFTP-17 fue definida en una conferencia de consenso en 1997 (Foster et al. 1997). La enfermedad fue descrita en 13 familias que presentaban una enfermedad hereditaria autosómica dominante. Esta enfermedad es un síndrome clínico poco frecuente que afecta aproximadamente a 200 familias y en las que unos 639 sujetos son portadores de mutaciones de *MAPT* (Spillantini, Bird, and Ghetti 2006). Los síntomas de presentación son demencia, desinhibición, parkinsonismo y amiotrofia. En estadios tempranos la disfunción de la memoria anterógrada no está presente y progresivamente aparecen disfunción global de memoria, alteración visuoespacial y desorientación. Los signos motores incluyen bradicinesia progresiva, rigidez axial e inestabilidad postural. Su inicio se sitúa hacia los 50 años de edad con un rango de presentación que oscila entre los 20 y los 70 años.

f. Enfermedad por Granos Argirófilos (DGA)

La DGA es una enfermedad neurodegenerativa esporádica, común de la edad senil y caracterizada por la presencia de granos argirófilos en la corteza entorrinal, hipocampo, amígdala y corteza temporal vecina. Es responsable de aproximadamente el 5% de todos los casos de demencia y puede estar asociada con otras enfermedades neurodegenerativas (EA, PSP, DCB y sinucleopatías como Cuerpos de Lewy (DCL), Enfermedad de Parkinson (EP) y Atrofia Multisistémica (AMS). La presentación clínica inicial es similar a la EA, pero la progresión de la enfermedad es menos agresiva, pudiendo manifestarse como deterioro cognitivo leve amnésico durante muchos años. La enfermedad también puede manifestarse como deterioro cognitivo y demencia con anomalías de comportamiento, personalidad y cambios emocionales. Estos casos apoyan la propuesta de considerar la DGA como una de las causas de DLFT. (Isidro Ferrer, Santpere, and van Leeuwen 2008).

2. GENOTIPOS. GENES MENDELIANOS Y DE SUSCEPTIBILIDAD ASOCIADOS A DLFT

La DLFT presenta un marcado componente familiar. Entre el 30-50% de los pacientes informan algún familiar con similar sintomatología y al menos un 10-30% se asocia a un patrón de herencia autosómica dominante. Sobreestimar o subestimar la tendencia genética dentro de los diferentes subtipos de DLFT y pensar en ella como una única entidad genética puede llevar a engaño. La DFTvc muestra una importante asociación familiar (45%), sobre todo cuando se asocia a la EMN (60%), mientras que la DS presenta una frecuencia muy baja (17%) de los casos familiares. (Goldman et al. 2005).

Al menos el 18% de las familias de DLFT con patrón de herencia autosómica dominante tienen una mutación del gen *Tau* en el cromosoma 17 (Rosso and van Swieten 2002). Otras familias se han vinculado a los cromosomas 3, 9, y a regiones no *Tau* del cromosoma 17. Otros síndromes con síntomas de DFT, incluyen la miopatía por cuerpos de inclusión asociada con la enfermedad de Paget y de DLFT causada por mutaciones en el gen de la Valosina (*VCP*).

Hasta ahora diez mutaciones han sido identificadas como causantes de DLFT, siendo las más frecuentes *MAPT, GRN* and *C9orf72*, en orden de descripción y publicación. En un grupo de 306 pacientes, se encuentra la expansión de *C9orf72* en un 8.2% de la muestra analizada y las mutaciones de *GRN* y *MAPT* en un 3.9% y 3.3% respectivamente (Wood et al. 2013).

a. Genes más comunes

MAPT: Microtubule associated protein Tau gen

La primera evidencia de una causa genética para la DLFT fue el hallazgo de la asociación del cromosoma 17q21.2 con la forma clínica autosómica dominante de DLFT familiar con parkinsonismo (Lynch et al. 1994). El gen responsable de la mutación, llamado *MAPT* fue descubierto en 1998 (Hutton et al. 1998). El gen *MAPT* codifica la proteína Tau asociada a microtúbulos, implicada en el ensamblaje y estabilización de los microtúbulos neuronales.

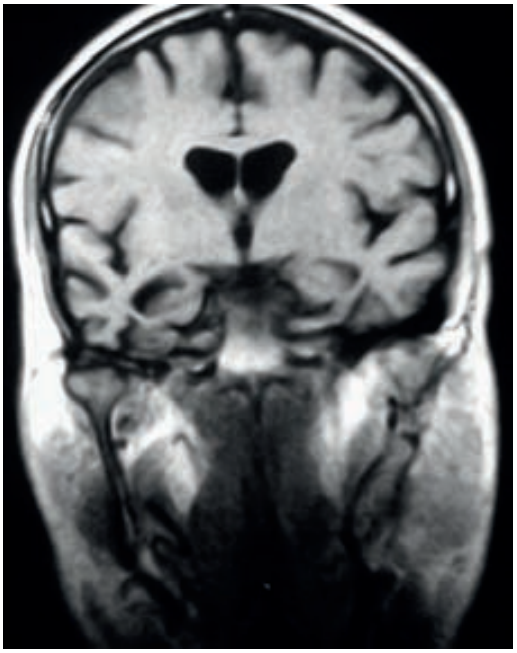
Hasta la fecha se han descrito 44 mutaciones *MAPT* patógenas en 134 familias. La frecuencia, según las series analizadas, varía entre un 1.9% y un 8.9% (J D Rohrer et al. 2009). No se ha identificado predilección de género y la edad de inicio se sitúa entre los 25 y 65 años (53±6) con un 100% de penetrancia. La evolución hasta el éxitus es de 3-10 años (Boeve and Hutton 2008).

Las mutaciones se agrupan principalmente en cinco exones (del 9 al 13), donde codifica los cuatro dominios de unión a microtúbulos de la proteína Tau. En el cerebro normal, la proteína Tau produce seis isoformas de las cuales tres contienen tres dominios (Tau 3R) y otras tres contienen cuatro dominios de unión a microtúbulos (Tau 4R). Un número considerable de mutaciones han sido localizadas en la región reguladora de corte y empalme del exón 10, dando lugar a proporciones aberrantes de Tau de 3R y 4R (Sieben et al. 2012)

La presentación clínica de los portadores de mutaciones *MAPT* es fundamentalmente DFTvc, aunque se han publicado casos de APP (Villa et al. 2011). Los síntomas incluyen disfunción ejecutiva y alteración de la personalidad y la conducta, evolucionando con afasia y parkinsonismo, en muchos casos, y mostrando una severa atrofia del lóbulo temporal. La asociación con ELA es rara. Fenotipos clínicos como EA, DS o SCB raramente se manifiestan.

La neuroimagen estructural muestra pérdida simétrica de volumen cerebral que engloba los lóbulos temporales anteromediales, cortex orbitofrontal y tractos de sustancia blanca, incluyendo el cuerpo calloso (Jonathan D Rohrer et al. 2010). Similar topografía puede observarse en el SPECT o PET-FDG. Los patrones de atrofia en pacientes portadores pueden ser heterogéneos pero afectan más comúnmente los lóbulos frontales y temporales, aunque la mayor atrofia se produce en el lóbulo temporal, en su mayoría derecho (K. a Josephs et al. 2009) (Whitwell and Josephs 2012). No se ha observado atrofia de cerebelo en estos pacientes (Whitwell et al. 2012).

La DLFT debida a mutaciones de *MAPT* se caracteriza patológicamente como Degeneración Lobar Frontotemporal con depósitos de proteína Tau (DLFT-TAU). Filamentos anormales de depósitos de Tau también se han descrito en otras enfermedades neurodegenerativas, incluyendo EA, DGA, PSP y DCB.



Paciente de la serie clínica de Fundació ACE, con mutación de MAPT y fenotipo DFTP-17, donde se evidencia la atrofia temporal derecha (Isidro Ferrer et al. 2003)

GRN: Progranulina

Otras mutaciones causantes de DLFT autosómica dominante, en un segundo gen del cromosoma 17q21, llamado Progranulina (*GRN*) fueron halladas en un serie de familias que previamente habían mostrado estar libres de mutaciones de *MAPT*. 63 mutaciones heterocigóticas han sido identificadas a nivel mundial en 163 familias, que explican alrededor del 5 al 10% de las DLFT (I Gijselinck, Van Broeckhoven, and Cruts 2008).

En los casos de mutaciones de *GRN,* el depósito de proteína predominante es una proteína ubiquitinada llamada "TAR DNA binding" (TARDBP or TDP-43) y los depósitos de Tau son raramente observados. La mayoría de mutaciones de *GRN* conocidas son mutaciones sin sentido que dan lugar a un codón de parada o empalme prematuro que altera la lectura del ARN mensajero (ARNm). El resultado es que el ARNm mutado se degrada por la descomposición mediada por la mutación y no se produce ninguna proteína a partir del gen mutado, produciendo la DLFT por una haploinsuficiencia de la *GRN* (Yu et al. 2010). El gen de la *GRN* está situado centromérico a 1.7Mb del

gen *MAPT*, en el cromosoma 17q21.31 y codifica un factor de crecimiento implicado en la regulación de varios procesos, incluyendo el desarrollo, la reparación de heridas y la inflamación. El gen también ha sido fuertemente asociado a la tumorogénesis. Además, la expresión de *GRN* se incrementa en la microglía activada de muchas enfermedades neurodegenerativas, incluyendo la enfermedad de Creutzfeldt-Jakob, ELA y EA (Baker et al. 2006).

Los fenotipos asociados a mutaciones de *GRN* varían ampliamente. Un 63% de los portadores desarrollan DFTvc y los demás pueden presentarse fenotípicamente como APP, SCB, DCL o EA. El 41% de los pacientes desarrolla parkinsonismo, el 25% alucinaciones visuales y el 24% apraxia motora. Los trastornos en la memoria episódica son frecuentes. Según los datos de un estudio francés (Le Ber et al. 2008) sobre una muestra de 502 sujetos, la frecuencia de mutaciones de *GRN* fue del 5.7% en el fenotipo DFTvc (17.9% de ellos con herencia autosómica dominante) 4.4% en fenotipo APP y el 3.3% en el fenotipo DCB. No se encontraron mutaciones en el fenotipo DFT-EMN. La neuroimagen muestra pérdida de volumen asimétrica, involucrando principalmente los lóbulos frontal inferior, temporal superior y parietal inferior, precuneo y cortex cingulado, así como tractos de sustancia blanca (Jonathan D Rohrer et al. 2010).

La frecuencia de mutaciones *MAPT* vs *GRN* varía según series y países. En Reino Unido 8.9% vs 8.4% (J D Rohrer et al. 2009) y 2.9% vs 4.8% (Pickering-Brown et al. 2008). En Francia 2.9% vs 4.8% (Le Ber et al. 2007), en USA 4.4% vs 4.8% (Gass et al. 2006) y en Bélgica 1.9% vs 10.7% (Cruts, Kumar-Singh, and Van Broeckhoven 2006).

Los pacientes con mutaciones de *MAPT y GRN* no difieren significativamente de otros casos de DLFT en términos de distribución por género. La historia familiar de demencia en primer grado está presente en el 100% de los casos de *MAPT*, 71% de los casos de *GRN* y en el 39% de otros casos de DLFT (Pickering-Brown et al. 2008) y la edad de inicio en los casos portadores de mutaciones de *GRN* es amplia (47-79 años), incluyendo los miembros de una misma familia (Pietroboni et al. 2011).

C9orf72: chromosome 9 open reading frame 72

Las mutaciones de *C9orf72* son la causa genética familiar y esporádica más común de DFTvc (11.7%) y ELA (23.5%). Es un gen de función desconocida hasta el momento, publicado al mismo tiempo en dos cohortes familiares de DFT y ELA en EEUU y Finlandia. (DeJesus-Hernandez et al. 2011), (Renton et al. 2011). La mutación fue particularmente frecuente en pacientes y familias con DFT-EMN. Se trata de una expansión

del hexanucleotido G_4C_2 no codificante en el gen *C9orf72*. En la población normal, el tamaño de la repetición G_4C_2 varía de 3 a 25 unidades, pero ésta se expande al menos 60 unidades en los pacientes afectos, pudiendo llegar a más de 1000. Sin embargo, no se ha encontrado ninguna asociación significativa entre el número de repeticiones, la forma de presentación fenotípica y la edad de inicio de la enfermedad (Rutherford et al. 2012).

En un extenso estudio realizado posteriormente en 17 regiones de todo el mundo, donde fueron incluidos 4448 pacientes con ELA y 1425 pacientes con DLFT, se han encontrado diferencias en la frecuencia de la expansión del *C9orf72* entre las regiones estudiadas (Majounie et al. 2012). Así, dentro de Europa la frecuencia más alta de mutaciones está presente en la población Finlandesa, con un 1.8% de DFT esporádicas (DLFTe), 21.1% de ELA esporádica (ELAe) y 46.5% de ELA familiar (ELAf), estando también presente la mutación en un tercio de los casos de ELA de descendientes consanguíneos europeos (Renton et al. 2011). La expansión de *C9orf72* está presente en alrededor del 6% de pacientes con ELAe en Alemania e Inglaterra, en 4.1% de los pacientes italianos con ELAe y en el 2.2% de los casos holandeses de DFTe. En los casos de población blanca de Australia y USA, un 5.0% de pacientes con ELAe también presentan la expansión.

Resumiendo, esta mutación explica una sustancial proporción de casos de ELAe (7.0%) y DFTe (6.0%) en la población blanca. La penetrancia relacionada con la edad muestra que el 50% de los portadores manifiestan la enfermedad hacia los 59 años de edad y que la mutación es totalmente penetrante a los 80 años (Majounie et al. 2012).

El consorcio "European Early Onset Dementia" (EOD) liderado por Chistine van Broeckhoven, también ha evaluado, en una muestra de 1205 pacientes, la distribución geográfica de la expansión *C9orf72* G_4C_2 en DLFT en Europa. La serie estaba formada por la cohorte de Flandes (Bélgica) y una cohorte europea de 15 países de Europa occidental (van der Zee et al. 2013). La frecuencia de la expansión C9orf72 fue del 9,98%: 18.52% en la forma familiar y 6,26% en pacientes esporádicos. Finlandia y Suecia mostraron la frecuencia más alta de la serie, con un 29,33% y 20,73% respectivamente, pero también España con 25,49%. La prevalencia en Alemania se limitó al 4,82%.

En la cohorte de Flandes-Bélgica (305 DLFT, 137 ELA, 23 DLFT-ELA y 856 controles) (Ilse Gijselinck et al. 2012) se ha observado que la expansión del *C9orf72* es altamente penetrante, con un 86% de frecuencia en DLFT-ELA familiar y 47% de ELA, y donde la

expansión es la causa genética más común y la única mutación identificada en el grupo de pacientes con DLFT-ELA. En este estudio el grupo DLFT con la expansión de la repetición G_5C_2 fue la segunda causa más común de enfermedad después de las mutaciones de *GRN*.

La frecuencia alélica de *C9orf72* también ha sido investigada en cuatro enfermedades neurodegenerativas (520 FTD, 289 ELA, 424 EA y 29 EP con la mutación *LRRK2 G2019S*) por el grupo de la Universidad de Toronto. La mutación fue detectada en el 9.3% de los pacientes con ELA, 7.2% de los pacientes con DFT y el 0.7% de los pacientes con EP, pero no en los controles ni en los pacientes con EA (Xi et al. 2012).

Aunque la mutación *C9orf72* parece ser una de las mutaciones más frecuentes asociada con DLFT, es difícil sospechar la presencia de esta mutación en parte debido a los numerosos casos esporádicos. El análisis de haplotipos de todos los pacientes (esporádicos y familiares) portadores de la mutación comparte el haplotipo de riesgo fundador finlandés. Estos hallazgos sugieren que esta mutación podría haber ocurrido hace unos 1500 años (una media de 100-105 generaciones) como un evento único y posteriormente diseminada al resto del mundo (Majounie et al. 2012).

Los fenotipos clínicos de presentación más frecuente incluyen DFTvc, ELA o DFT-ELA. Muchos casos con el fenotipo predominante DFTvc pueden tener implicación de neurona motora superior y/o inferior y algunos con fenotipo ELA pueden presentar signos característicos de DFTvc. Los fenotipos APP y SCB no aparecen asociados a esta mutación. La psicosis y los cambios en la conducta alimentaria son comunes. Ansiedad y agitación pueden ser precoces, prominentes y de suficiente significación, en algunos casos, como para contactar tempranamente con los servicios de psiquiatría (Boeve et al. 2012) (Mahoney et al. 2012).

Los signos neuropsicológicos revelan alteración en la atención, funciones ejecutivas y fluencia verbal. El rendimiento en otros dominios es muy variable (Boeve et al. 2012). El trastorno de memoria domina la presentación clínica en algunos casos, dando lugar al diagnóstico inicial de EA (Mahoney et al. 2012).

La neuroimagen muestra perdida cortical extensa, relativamente simétrica en los dos hemisferios e involucrando a lóbulos frontales, temporales y parietales. El análisis morfométrico basado en voxels muestra pérdida de sustancia gris en tálamo y cerebelo, lo que podría explicar las características neuropsiquiátricas prominentes en estos casos, como el déficit de memoria episódica, quejas somáticas, alucinaciones y delirios (Tedesco et al. 2011).

b. Otros genes menos comunes asociados con DFT hereditaria

VCP: valosin-containing protein

Las mutaciones del *VCP* fueron identificadas en el cromosoma 9p21.1 a través de estudios de análisis de ligamiento en familias con herencia autosómico dominante. Los sujetos afectos de dichas familias presentaban debilidad muscular incapacitante debida a miopatía por cuerpos de inclusión, lesiones óseas osteolíticas compatibles con enfermedad de Paget y DFTvc (Inclusion Body Miopathy, Paget and Frontal Dementia - IBMPFD) (Watts et al. 2004). Los investigadores encontraron 6 mutaciones sin sentido en el gen codificante de la proteína "valosin-containing" (VCP), exclusivamente en los 61 individuos afectados. El análisis de haplotipos indicaba que los descendientes de los fundadores, en 2 familias no relacionadas de Norteamérica, suponían más o menos el 50% de las familias afectadas. El gen de la *VCP* codifica una proteína de la superfamilia de las AAA-ATPasas que facilita la degradación de las proteínas por las vías de la ubiquitina-proteasoma y la autofagia. Los tejidos de los cerebros y músculos afectados en IBMPDF presentan depósitos ubiquitinados e inclusiones de TAR-DNA binding protein-43 (TDP-43) (Weihl 2011).

Clínicamente, el 90% de los pacientes desarrollan debilidad muscular incapacitante a una edad media de 45 años. Hacia la misma edad, el 51% de los pacientes desarrollan enfermedad de Paget y el 32% desarrollan trastornos de conducta y lenguaje con una media de edad de 54 años (Kimonis & Watts , 2005). Sólo el 3% presentan DFTvc como fenotipo aislado (Kimonis et al. 2008). Han sido comunicadas otros síntomas, como cardiomiopatía dilatada, esteatosis hepática, cataratas y neuropatía axonal sensitivo-motora. Lo pacientes no siempre expresan los tres componentes del fenotipo, pudiendo expresar uno o dos aisladamente (Guyant-Maréchal et al. 2006).

CHMP2B: Charged multivesicular body protein

La mutación *CHMP2B* en el cromosoma 3p11.2 fue identificada por análisis de ligamiento en una familia Danesa con miembros afectos de DLFT (Skibinski et al. 2005). *CHMP2B* es un componente del complejo ESCRT-III, que se requiere para la función de los cuerpos multivesiculares (MVB), una estructura endosomal que se fusiona con el lisosoma para degradar las proteínas por endocitosis. Los estudios funcionales demuestran una alteración específica de la fusión endosoma-lisosoma, necesaria para la correcta función neuronal (Urwin et al. 2010).

El fenotipo característico es DFTvc, con cambios precoces de personalidad como síntoma principal, acompañado de hiperoralidad e ingestión de objetos no comestibles. Los pacientes afectos presentan desinhibición marcada y respuestas emocionales inadecuadas. La edad de inicio varía entre 46 y 65 años (media de 57 años) en el pedigrí comunicado por Gydesen (Gydesen et al. 2002) y seguidos durante los 17 años del estudio. El patrón de herencia es autosómico-dominante con alta penetrancia y observándose anticipación genética en los casos de transmisión paterna. Otras mutaciones en *CHMP2B* (Q206H y 129V) han sido identificadas en 2 pacientes con ELA y negativas para otras formas conocidas de mutaciones de ELA. (Parkinson et al. 2006). La neuropatología muestra pérdida neuronal y gliosis sin características histopatológicas específicas.

TARDBP: TAR DNA Binding Protein

El gen *TARDBP* proporciona instrucciones para la fabricación de la proteína "transactive response DNA binding protein 43 kDa" (TDP-43). Esta proteína se encuentra dentro del núcleo de la célula en la mayoría de los tejidos y está implicada en muchos de los pasos de la producción de proteínas.

Las mutaciones de *TARDBP* fueron identificadas inicialmente como consecuencia directa de la identificación de la proteína TDP-43 como la mayor constituyente de los agregados observados en las DLFT ubiquitin positivas (DLFT-U) y en las neuronas motoras superiores e inferiores de los pacientes con ELA sin mutaciones *SOD1*. (Sreedharan et al. 2008). Alrededor del 5% de los pacientes con ELA familiar tienen la mutación *TARDBP* y es raramente hallada en la DFT-ELA (Benajiba et al. 2009).

FUS: Fused in sarcoma

Las mutaciones de *FUS* han sido comunicadas como causantes del 4% de los casos de ELA familiar (Vance et al. 2009) (Kwiatkowski et al. 2009). Hasta hoy 15 diferentes mutaciones de *FUS* han sido descritas en 26 ELA familiares no relacionadas.

El gen *FUS* está localizado en el cromosoma 16p11.2 y codifica la proteína FUS, miembro de un heterogéneo grupo de ribonucleoproteinas nucleares (hnRNP family). Estas proteínas comunican información crucial para la maquinaria de traducción y localización del RNAm y la vigilancia de las "nonsense mutations" (Dreyfuss, Kim, and Kataoka 2002).

Mutaciones de sentido erróneo de *FUS* han sido también identificadas en paciente con DFTvc pura, lo que ha llevado a sugerir que ELA y DLFT son parte del mismo espectro clínico, genético y patológico (Van Langenhove et al. 2010). La patología subyacente está caracterizada por depósitos de proteína FUS positivos e inclusiones TDP-43 negativas.

TARDBP y *FUS* tienen una estructura y funcionalidad similar y muchas de las mutaciones en ambos genes también se agrupan en el extremo C-terminal de las proteínas. Los mecanismos moleculares a través de los cuales las TDP-43 y FUS mutadas pueden causar la degeneración de las neuronas motoras no están bien aclarados. Ambas proteínas juegan un importante papel en el transporte de RNAm, el mantenimiento axonal y el desarrollo de las neuronas motoras.

UBQLN2: Ubicuilin 2

Mutaciones en el gen de la *UBQLN2*, que codifica una proteína ubiquitinada llamada ubiquilin 2 causan ELA y ELA-demencia de causa autosómica dominante ligada al CrX. Cinco mutaciones diferentes en el gen *UBQLN2* han sido identificadas en DFT-ELA, ligadas al cromosoma X pero no totalmente penetrantes (Deng et al. 2011), encontrando también estos autores, correlación de la patología *UBQLN2* hipocampal con demencia en los casos de ELA con o sin mutaciones *UBQLN2*, lo que sugiere que este gen podría estar implicado en la demencia relacionada con la ELA, incluso sin mutaciones *UBQLN2*.

La proteína ubiquitina 2 es un miembro de la familia de las ubicuilinas, que regulan la degradación de las proteínas ubiquitinadas. El papel de la ubiquitinización en neurodegeneración ha sido bien establecido en diferentes enfermedades neurodegenerativas humanas como la enfermedad de Parkinson (Leroy et al. 1998) o el Síndrome de Marinesco-Sjögren (Zhao et al. 2010). Inspecciones preliminares de secuencias no-PXX en 130 casos de ELA familiar en una serie francesa (Millecamps et al. 2012) y en 77 casos de DFT con historia familiar positiva en Cataluña (Hernández et al. 2012), sugieren que las mutaciones en la línea germinal de *UBQLN2* son escasas, tanto en DFT como en ELA familiares.

TREM2: Triggering receptor expressed on myeloid cells 2

El gen TREM2 proporciona instrucciones para fabricar una proteína llamada "*triggering receptor expressed on myeloid cells 2*", proteína transmembrana tipo I que interacciona con la proteína formada por el gen *TYROBP* llamada "*tyrosine kinase- binding protein*". Ambas proteínas, TREM2 y TYROBP forman un complejo que transmite señales químicas para la activación de la respuesta inmune en macrófagos y células dendríticas. *TREM2* se expresa en macrófagos y células dendríticas pero no en granulocitos o monocitos, sugiriendo el rol de *TREM2* más bien en los estados crónicos que en los inflamatorios (http://omim.org/entry/605086).

Mutaciones en ambos genes han sido asociadas con la "osteodisplasia poliquística lipomenbranosa con leucoencefalopatia esclerosante" (PLOST) o enfermedad de Nasu-Hakola. Esta enfermedad está asociada con fracturas patológicas por lesiones óseas poliquísticas (de inicio en la tercera década de la vida) seguido de cambios progresivos de conducta y personalidad (consistentes con DFTvc) en la siguiente década (Paloneva BM et al. 2001). En tres de 44 pacientes turcos con diagnóstico clínico de DFT-like, fueron identificadas en *TREM2* diferentes mutaciones homocigóticas, que habían sido previamente asociadas a cambios de conducta y deterioro cognitivo con signos motores pero sin los fenotipos ni quistes óseos asociados con PLOST.

Un estudio reciente llevado a cabo por "*The Dementia Genetic Spanish Consortium*" (DEGESCO) (Ruiz et al. 2014) evaluando el papel del polimorfismo p.R47H del gen *TREM2* en 3172 EA, 682 DLFT y 2169 controles sanos, como factor de riesgo para la EA y la DLFT, concluye que el 0.6% de los pacientes EA son portadores de esta variante, comparado con el 0.1 % de los controles [OR]= 4.12 sugiriendo que esta rara variante no está relacionada con la DLFT pues no apareció en ningún caso estudiado.

Otro estudio reciente llevado a cabo en la Universidad de Bonn y en colaboración con DEGESCO (Thelen et al. 2014) para detectar otras variantes raras de TREM 2 en los diferentes subtipos de DFT-S (DFT síndromes), ha identificado 7 variantes raras (p.A28V, p.W44X, p.R47H, p.R62H, p.T66M, p.T96K, y p.L211P) y una nueva mutación de cambio de aminoácido (p.A105T). La variante p.R47H fue hallada en 4 pacientes con diagnóstico clínico de DFT-S pero dos de ellos mostraban bioquímica el LCR típica de EA, lo que sugiere que estos pacientes presentan fenotipo DFT-S pero una patología EA subyacente. No se encontró asociación con esta variante en DFT. Si se asociaron a DFT las variantes p.T96K y p.L211P. No se encontró alteración en ninguno de los controles.

SQSTM1: Sequestrosoma 1

SQSTM1 codifica la proteína P62 (también llamada sequestrosoma 1) una nueva proteína de unión a ubiquitina que tiene un importante rol en la degradación de proteínas vía proteasoma y autofagia, siendo importante esta última vía en la digestión lisosomal de los constituyentes celulares. La acumulación de la P62 no es específica para la ELA y la DLFT, habiéndose hallado también en la Enfermedad de Alzheimer, Parkinson, Atrofia Multisistémica y enfermedad de Pick. También ha sido observada en inclusiones citoplasmáticas TDP-43, ubiquitina y UBQLN2 en pacientes DLFT con ELA (Appel and Rowland 2012). Recientemente se han identificado mutaciones en los casos familiares y esporádicos de ELA (Teyssou et al. 2013).

En un estudio reciente se ha analizado la secuencia de codificación para las mutaciones de SQSTM1 en una cohorte de 1.808 pacientes con DLFT procedentes del consorcio EOD. (van der Zee et al. 2014). Como controles se han utilizado 1.625 individuos europeos y se han analizado los datos de todo el exoma de 2274 individuos alemanes (total n = 3899). Se ha realizado también en un meta-análisis de 4332 FTLD y 10.240 alelos de control la asociación con SQSTM1. El trabajo ha identificado 25 variantes en la región codificante de *SQSTM1* en pacientes con DLFT, de las cuales 10 eran nuevas. Quince mutaciones estaban ausentes en los controles (frecuencia portadores <0,00026), mientras que las otras eran poco frecuentes en ambos (pacientes y controles). El estudio ha demostrado que las mutaciones *SQSTM1* están asociadas a la patología TDP-43.

c. Genes de susceptibilidad

TMEM106B: transmembrane protein 106B y otros

Para buscar nuevos genes asociados a DLFT, un equipo internacional liderado por la Facultad de Medicina de la Universidad de Pennsylvania (USA), realizó en 2010 un estudio amplio de asociación del genoma (GWAS) en 515 pacientes DLFT con inclusiones de TDP-43 confirmadas en autopsia y 2509 controles poblacionales (Deerlin et al. 2010). Todos los casos cumplían criterios genéticos o patológicos para DFT-TDP, lo que fue confirmado por la detección de mutaciones o de inclusiones TDP-43 mediante inmuno-histoquímica. Los autores encontraron, tras el mapeo en el Cr.7p21, múltiples SNPs asociados a DFT-TDP. Esta región contiene el gen *TMEM106B*. Tres SNPs mostraban asociación genética después de múltiples pruebas de corrección; el alelo menor (C) del SNP rs1990622 se mostró como factor genético de protección para DLFT-TDP. La asociación se replicó en 89 casos autopsiados de DFT-TDP y 553 controles, pero no en una serie clínica de 192 DFT.

Estudios independientes intentando replicar estos hallazgos en series clínicas han mostrado resultados controvertidos. Así, en la cohorte clínica de Londres y Manchester (520 casos de DFT y 247 controles) no se encontró ninguna asociación en *TMEM106B* (Rollinson et al. 2011). Sin embargo, usando la cohorte de Flandes (Bélgica), compuesta principalmente por pacientes clínicos (288 DFT y 595 controles) se confirmó la asociación con *TMEM106B* rs1990622 [OR=0.75 (0.61–0.93)] (van der Zee et al. 2011).

Los autores identifican *TMEM106B* como un factor de riesgo importante para la DLFT con patología TDP-43. La asociación más significativa ha sido observada en aquellos casos con mutaciones de *GRN* (Lang et al. 2012).

Se ha realizado otro GWAS analizando un total de 3.526 casos y 9402 controles de descendientes europeos (Ferrari et al. 2014).

Los distintos fenotipos clínicos de DFT se analizaron por separado, con los siguientes resultados:

► El locus *TOMM40/APOE* superó la significación estadística en la DFTvc, pero no en DS, APNF y DFT-ELA. Esta asociación puede venir a reflejar una contaminación (~15%) de EA en casos clínicamente diagnosticados de DFT.

► En el locus *TMEM106B* el estudio sólo mostró una discreta asociación en DFTvc ($P = 0.00585^{)}$, pero no se encontró asociación con los otros subtipos de DFT. En este estudio los portadores de mutaciones de *GRN* fueron excluidos.

Vale la pena señalar que la publicación original incluía un número de portadores de mutaciones GRN (Deerlin et al. 2010) y que la evidencia bioquímica ha sugerido que *TMEM106B* está directamente relacionado con el metabolismo de GRN.

3. PROTEOTIPOS DLFT

Múltiples son las anormalidades neuropatológicas asociadas a la DLFT. Las características clínicas y neuropsicológicas ayudan a orientar el posible espectro patológico en el paciente diagnosticado de DFT, pero son los biomarcadores, junto con el fenotipo clínico los que van a predecir la neuropatología subyacente. Todas la DLFT están asociadas a grados variables de atrofia, pérdida neuronal y gliosis en los lóbulos frontales y temporales. Sin embargo, cada enfermedad difiere una de otra por las diferencias en el depósito de proteínas, la firma bioquímica y la morfología y distribución de las inclusiones.

Tres proteínas principales han sido identificadas en el mecanismo de la neurodegeneración de las DLFT. Éstas son la "*microtubule-associated protein*" (Tau) (Hutton et al. 1998), la "*transactive response DNA binding protein of 43 kD*" (TDP-43) (Neumann et al. 2006)(Arai et al. 2006), y la "*tumor associated protein fused in sarcoma*" (FUS) (Kwiatkowski et al. 2009). Algunos de los más renombrados neuropatólogos (I R A Mackenzie et al. 2009) recomiendan que la terminología fenotípica de las DLFT debe conservar las nomenclatura asociada a las entidades clínicas, como DFT, APNF y/o DS, mientras que las subdivisiones neuropatológicas deben designarse por la proteína anómala patógena más característica que ha dado lugar al proceso clínico (es decir DLFT-proteína). Por lo tanto, en el estrato más alto, la mayoría de las DLFT deben ser sub-clasificadas en DFLT-Tau, DLFT-TDP y DLFT-FUS.

(Tabla 1, pag 49)

a. DLFT-TAU

La proteína Tau (proteína asociada a microtúbulos) se acumula tanto en neuronas como en células gliales. En casos esporádicos los acúmulos pueden formar cuerpos de Pick en neuronas (DLFT-Tau [enfermedad de Pick]), astrocitos estrellados (DLFT-Tau [Parálisis Supranuclear Progresiva]) o placas astrocíticas (DLFT-Tau [Degeneración Cortico-Basal]), mientras que en los casos hereditarios, estos pueden presentarse como inclusiones similares a una de ellas o como patología Tau única (Halliday et al. 2012).

Alrededor del 40% de los pacientes con DLFT muestran inclusiones Tau. Estos incluyen la mayoría de los casos de APNF, ~45% de los casos de DFTvc y algunos casos de DS (Piguet et al. 2011). Crecientes evidencias sugieren que la presencia de estas lesiones neurofibrilares en la fase final de la enfermedad no son la causa de la pérdida neuronal, sino que la neurodegeneración está provocada por la alteración de la proteína Tau soluble. En particular, la fosforilación Tau aberrante es reconocida como clave en el proceso de la enfermedad, influyendo en la estructura de Tau, su distribución y su función en las neuronas (Noble et al. 2013).

La proteína Tau es el componente principal de los ovillos neurofibrilares observados en los cerebros de pacientes con enfermedad de Alzheimer y otras enfermedades neurodegenerativas. Es una proteína altamente soluble que se encuentra predominantemente en las neuronas. Seis isoformas diferentes de Tau son expresadas en el sistema nervioso adulto gracias a los procesos de maduración alternativa del gen MAPT, que comprende 16 exones y se encuentra en el cromosoma 17q21.3. La inclusión regulada de los exones 2 y 3 dan lugar a isoformas de Tau con 0, 1 ó 2 inserciones N-terminal, mientras que la exclusión o inclusión del exón 10 conduce a la expresión de isoformas de Tau con tres (3R) o cuatro (4R) repeticiones de unión a microtúbulos. En el cerebro humano normal la proporción de 4R-3R es aproxima-damente de uno, mientras que en muchas taupatías la proporción está alterada. Así, la PSP, la DCB y la enfermedad por granos argirófilos (EGA) presentan una sobre-expresión de isoformas Tau de 4R, mientras que la enfermedad de Pick esta principalmente caracterizada por inclusión de isoformas Tau de 3R (Noble et al. 2013).

b. DLFT-TDP

Más del 50% de los pacientes con DLFT que presentan una tinción negativa para inclusiones Tau, pero son positivas para tinciones de Ubiquitina y el 80-90% de este grupo está compuesto por inclusiones TDP-43 ("*transactive response* (TAR) *DNA-binding protein 43*") (Roeber et al. 2008). TAR DNA-binding protein 43 (TDP-43) es una de las principales proteínas de la DLFT con inclusiones ubiquitín-positivas (DLFT-U), Tau-negativas con o sin enfermedad de motoneurona.

TDP-43 es una proteína de unión al ADN nuclear implicada en la regulación transcripcional y que se deposita de forma aberrante en inclusiones citoplásmicas filamentosas después de una serie de modificaciones post-traducción, incluyendo proteólisis, fosforilación y ubiquitinación (Neumann et al. 2006).

Actualmente son reconocidos cuatro subtipos de DLFT-TDP. La clasificación de estos subtipos está basada en el aspecto morfológico de las inclusiones y en la distribución de las lesiones (I. R. a Mackenzie, Neumann, et al. 2011).

► Tipo A, caracterizado por numerosas neuritas distróficas cortas (DN) e inclusiones citoplasmáticas ovales semilunares (NCI) que se concentran principalmente en la capa 2 neocortical. Son también características comunes, pero inconsistentes de este subtipo, un moderado número de inclusiones neuronales lentiformes (NII).

► Tipo B, caracterizado por un moderado número de NCI a lo largo de todas las capas corticales, pero muy pocas DN.

► Tipo C, con predominio de DN alargadas en las capas corticales superiores con muy pocas NCI.

► Tipo D, se asocia a la patología IBMPFD, causada por mutaciones de VCP y que se caracteriza por DN numerosas y cortas y frecuentes NII lentiformes.

La patología TDP-43 también se ha encontrado en el lóbulo temporal en el 23% de los casos de EA y el 71% de los casos de esclerosis hipocampal (Amador-Ortiz et al. 2007). Así mismo, la patología TDP-43 comórbida ha sido identificada en el 29% de los casos de enfermedad por Cuerpos de Lewy y EA, en el 19% de los casos de Parkinson-Demencia y en el 7% de le enfermedad de Parkinson (Nakashima-Yasuda et al. 2007).

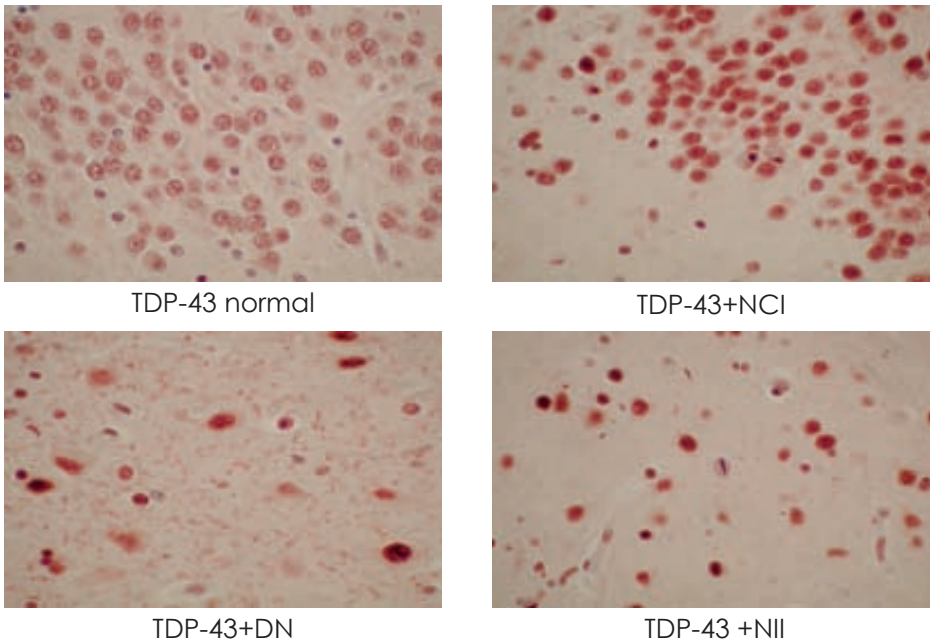


Figura 1. Diferentes subtipos de TDP- 43

Imágenes cedidas por el Dr. Ronald M. Hamilton. (Associate Professor of Neuropathology, University of Pittsburgh School of Medicine). Bienal Barcelona-Pittsburg 2008.

c. DLFT-FUS

Como se ha visto hasta ahora, muchos casos de DLFT están caracterizados por una acumulación intracelular anormal de proteínas Tau o TDP-43, pero más del 10% de los casos están compuestos por una acumulación heterogénea de depósitos poco comunes. El 10-20% de DLFT no muestran evidencia de TDP-43 anormal y algunos subtipo de DLFT Tau/TDP-43 negativos son inmunoreactivos (ir) para la proteína "*fused in sarcoma*" (FUS) (I. R. a Mackenzie, Muñoz et al. 2011).

La categoría DLFT-FUS hace mención a tres enfermedades relativamente raras: la enfermedad por inclusión de filamentos intermedios (NIFID), la enfermedad por inclusión de cuerpos basofílicos (BIBD) y la DLFT atípica con cambios inmunoreactivos sólo para ubiquitina (aDLFT-U). Estas tres entidades comparten el hecho de que todas muestran inmunoreactividad para FUS, pero las características encontradas entre cada una de ellas permite que sean consideradas entidades patológicas diferentes (K. A. Josephs et al. 2011).

► NIFID: DFT esporádica de presentación típica en edad temprana, asociada a signos piramidales y/o extrapiramidales. Los hallazgos inmunohistoquímicos comunes en todos los casos son inclusiones de alfa-internexina y de neurofilamentos positivos citoplasmáticos, sin las densidades comparables a otras enfermedades neurodegenerativas (Roeber et al. 2006).

► BIBD: término usado para un pequeño número de casos clínicos y patológicamente heterogéneos que tienen en común el hallazgo de NCI que son basofílicos para las tinciones de hematoxilina y eosina (inclusiones basofílicas [BI]). Los fenotipos clínicos incluyen ELA esporádica, ELA familiar, ELA con demencia y DFT pura (I. R. a Mackenzie, Munoz, et al. 2011).

En ambas patologías los signos iniciales pueden incluir: debilidad y alteración de memoria en BIBD y disartria en ambas. En alguno de los casos, la demencia de desarrolla más de un año después del inicio de los síntomas. Se han observado signos de neurona motora superior e inferior, parkinsonismo y signos parietales en ambas enfermedades. También aparecen movimientos involuntarios en BIBD. Un hallazgo consistente en las dos patologías es la severa atrofia de caudado (Yokota et al. 2008).

► aDLFT-U: Es un fenotipo clínico esporádico e inusual de inicio temprano (media de 35 años). Se caracteriza por una rápida y severa alteración conductual en ausencia de déficits de lenguaje o motores significativos (Ian R A Mackenzie et al. 2008). La neuropatología es también atípica, mostrando NCI y NII que

aparecen como filamentos largos y gruesos, que pueden ser rectos, curvos o sometidos a torsión (vermiformes). Los NCI y los NII en la aDLFT-U son sistemáticamente inmunoreactivos con anticuerpos contra FUS (Neumann et al. 2009).

En aproximadamente el 50% de los casos de NIFID y BIBD se encuentra una degeneración de motoneurona, pero ésta no ha sido comunicada en la aDLFT-U (K. A. Josephs et al. 2011).

d. DLFT-UPS

DLFT-UPS se designan a aquellos casos con inclusiones que sólo se pueden demostrar inmuno-histoquímicamente contra el sistema ubiquitina proteasoma (UPS) (I R A Mackenzie et al. 2009).

La DFT-3 (mutaciones en el gen CHMP2B) es la principal entidad asociada con la patología DLFT-UPS. Esta designación reconoce que las inclusiones TDP-43 negativas pueden presentar inmunotinción para proteínas UPS diferentes a la ubiquitina, como la p62 (SQSTM1).

El examen macroscópico muestra una atrofia cortical generalizada, más acusada en las cortezas frontal y temporal. La microscopía muestra pérdida de neuronas corticales, microvacuolación de la capa II, gliosis leve y desmielinización de la sustancia blanca profunda. Inclusiones raras ubiquitina-positiva también se encuentran en neuronas frontales y temporales corticales, positivas para p62, pero no para TDP-43 (Holm et al. 2007).

La proteína p62 es codificada por *SQSTM1* (también llamado sequestrosoma 1), La acumulación de p62 no es específica de ELA y DLFT. Inclusiones ubiquitina-positivas conteniendo p62 ha sido comunicada en EA, EP, Atrofia Multisistémica y enfermedad de Pick. La p62 también se puede encontrar asociada a inclusiones citoplasmáticas TDP-43 (+), ubiquitina y en pacientes con DLFT con EMN por mutaciones de *UBQLN2*. La presencia de p62 en las inclusiones ubiquitina (+) en diferentes enfermedades neurodegenerativas, centra la atención en un tema común de importancia potencial en estos trastornos. Es decir, el papel en los procesos neurodegenerativos de las proteínas mal plegadas, la deficiente digestión proteosómica y la autofagia (Appel and Rowland 2012).

Tabla 1. Clasificación molecular, fenotipos y genes asociados en la DLFT.

Clasificación Molecular	Inclusiones patológicas	Subtipo patológico	Fenotipo Clínico asociado	Genes asociados y localización
DLFT-Tau	Tau (+)	PiD (3R)	DFTvc	*MAPT* (17q21.1)
		DCB (4R)	APNF	
		PSP (4R)	APNF	
		EGA (4R)	DFTvc	
		NFT-demencia (3+4R)	DFTvc	
		MSTD (4R)	DFTvc	
DLFT-TDP	TDP-43(+), NCI, DN	Tipo A	DFTvc APNF	*GRN* (17q21.23)
	TDP-43(+), p62(+), NCI, DN	Tipo B	DFTvc EMN con DFT	*C9orf72* (9p21.2)
	TDP-43(+), NCI, DN	Tipo C	DS DFTvc	
	TDP-43 (+), NCI, NII, DN	Tipo D	IBMPFD Familiar	*VCP* (9p13.3)
	TDP-43 (+)		ELA	*TARDBP* (1p36.22)
DLFT-FUS	Ubicuitin(+) TDP-43 (-), NCI, NII	NIFID BIBD aDLFT-U	DFTvc	*FUS* (16p11.2)
DLFT-otras	Ubicuitin (+), TDP-43 (-), NCI	DLFT-ni DLFT-UPS	DFTvc	*CHMP2B* (3p11.2)

DLFT-Tau: DLFT caracterizada por inclusiones Tau inmunoreactivas, DLFT-TDP: DLT caracterizada por inclusiones TDP-43 inmunoreactivas. DLFT-FUS: DLFT caracterizada por inclusiones FUS inmunoreactivas, DFLT-otras: DLFT inclasificables (inclusions inmunoreactivas no Tau, no TDP-43, no FUS), Tau(+): Inclusiones Tau positivas, TDP-43(+): Inclusiones TDP-43 positivas, NCI: Inclusiones neuronales citoplasmáticas, DN: Neuritas distróficas, NII: Inclusiones intraneuronales, p62(+): Proteína de unión a ubiquitina, Ubicuitin (+) : Ubiquitina, 3R: Tres repeticiones , 4R: Cuatro repeticiones, PiD: enfermedad de Pick, DCB: Degeneración Cortico-Basal, PSP: Parálisis Supranuclear Progresiva, EGA: Enfermedad por granos argirófilos, NFT-demencia: Demencia por Ovillos Neurofibrilares, MSTD: Taupatía multisistémica esporádica, DFTvc: Demencia Frontotemporal variante de conducta, APNF: Afasia Progresiva no fluente, EMN: Enfermedad de Motoneurona, DS: Demencia Semántica, SCB: Síndrome Corticobasal, IBMPFD: Miopatía por cuerpos de inclusión con enfermedad de Paget y DFT, NIFID: Enfermedad por inclusión de filamentos neuronales intermedios, BIBD: Enfermedad por cuerpos de inclusión basofílicos , aDLFT-U: DLFT atípica con inclusiones de ubiquitina, FTLD-ni: DLFT sin inclusiones, DLFT-UPS: DLFT con inmuno-histoquímica contra proteínas del sistema proteosomal de la ubiquitina, MAPT: Gen de la proteína Tau asociada a microtúbulos, GNR: Progranulina, C9orf72: Cr9 open reading frame 72, VCP: valosin-containing protein, TARDBP: TAR DNA-binding protein, FUS: Fused in sarcoma, CHMP2B: Charged multivesicular body protein 2B.

IV. Hipótesis

Las enfermedades neurodegenerativas en general y la demencia Frontotemporal en particular tienen una importante base genética. Desde un punto de vista estrictamente genético, la heterogeneidad alélica y no alélica son la norma de estas enfermedades. Ello implica que existe cierta convergencia anátomo-patológica y clínica de alteraciones genéticas muy diversas y que afectan a funciones neuronales radicalmente distintas. La identificación de patrones clínicos o fenotípicos con las diferentes mutaciones de base es una tarea compleja que requiere grandes series epidemiológicas bien fenotipadas y un análisis molecular muy preciso.

Durante este trabajo de investigación se profundiza en el análisis de los perfiles clínicos y evolutivos de las demencias frontotemporales y su correlación con el genotipo identificado en los pacientes.

V. Objetivos

Al diseñar este trabajo se ha tenido en cuenta la gran variabilidad genética de la DLFT. Por tanto, su abordaje siempre se ha de enmarcar bajo un prisma multicéntrico y multidisciplinar. Nuestro objetivo siempre ha sido abordar los estudios concretos que se han planteado sin que éstos se solapen con los trabajos de los diferentes grupos de investigación con los que ya se colaboraba. A estos centros se les ha cedido parte de las muestras disponibles de la serie clínica para que realicen estudios en otros loci. Estas colaboraciones han generado diversas publicaciones (ver Anexo) y complementan el estudio genético de los pacientes disponibles en la institución. Teniendo en marcha numerosas colaboraciones, se optó por abordar los genes más recientes y menos conocidos al diseñar los objetivos de este trabajo de tesis (*UBQLN2*, *C9orf72* y *TMEM106B*).

Así, los objetivos planteados han sido los siguientes:

1. Analizar la prevalencia de mutaciones en el locus *UBQLN2* en pacientes con historia familiar de demencia frontotemporal dentro de la serie clínica aportada y realizar la correlación fenotipo-genotipo de las mutaciones identificadas.

2. Estudiar la prevalencia de la expansión del locus *C9ORF72* en la serie clínica aportada para posteriormente realizar la correlación fenotípica en los portadores de la mutación.

3. Realizar una correlación genotipo-fenotipo de la serie clínica de DFT, usando el polimorfismo *ApoE* como elemento discriminante para identificar sujetos con posible Enfermedad de Alzheimer (EA) de predominio frontal, que puedan estar contaminando la serie clínica.

4. Estudiar la presencia del polimorfismo de riesgo del SNP rs1990622 del locus *TMEM106B* en pacientes con fenotipo DFT, excluyendo de la serie clínica aquellos sujetos con confirmación anatomopatológica de proteotipo Tau, para posteriormente realizar la correlación fenotípica.

VI. Material y métodos

La metodología empleada en la presente tesis doctoral se explica con detalle en el correspondiente apartado de los trabajos de investigación presentados. En este apartado tan sólo se describen las características de la serie utilizada, recogida en su totalidad en Fundació ACE.

De un total de 17.042 sujetos valorados en la Unidad de Memoria de Fundació ACE, en primera visita (periodo 1996-2012), 9102 (53%) cumplían criterios de demencia según los diferentes criterios internacionales y 5218 (31%) criterios de Deterioro Cognitivo Leve. De los sujetos diagnosticados de demencia, 495 (5,4%) cumplían criterios de DFT posible o probable en sus diferentes variantes fenotípicas.

Para este trabajo de tesis se han seleccionado 224 (45.2%) de los 495 sujetos diagnosticados de DFT, ya fuera en primera visita o que durante el seguimiento clínico modificaran su fenotipo hacia DFT y que dispusieran de suficientes variables clínicas evolutivas y material biológico. Todos ellos disponían de valoración neurológica y neuropsicológica, y en 180 (80.3%) se ha podido rescatar la neuroimagen histórica para su posterior valoración.

La valoración de DFT por neuroimagen la llevaron a cabo dos neurólogos clínicos de la Unidad de Memoria ciegos para el fenotipo inicial y evolutivo de los sujetos y clasificándolos en 6 patrones de atrofia cerebral.

La serie anatomopatológica disponible ha sido posible gracias a las donaciones de los familiares de los pacientes y en colaboración con el Banco de Tejidos Neurológicos de la Universidad de Barcelona (IDIBAPS).

La muestra de sangre para los estudios genéticos, su obtuvo directamente de los pacientes. Se registró la firma de un consentimiento informado por parte del paciente o de sus representantes legales, de acuerdo con el protocolo GIPSY aprobado por el comité ético del Hospital Clínico y Provincial de Barcelona. El consentimiento informado está en consonancia con las leyes biomédicas españolas (Ley 14/2007, 3 de julio, de investigación biomédica y Real decreto 1716/2011, de18 noviembre).

Tabla 2. Demografía de la serie clínica DFT en Fundació ACE.

Diagnóstico Basal	Demografía N (%)						
	N (%)	Varón (%)	Educación >6 años (%)	Años de evolución al diagnóstico (x̄)	Años al diagnóstico (x̄)	Historia familiar N (%)	MMSE (x̄)
Psiquiátrico	3(1.3)	33.3	33.3	6±7.1	70.3±4.9	2 (66.7)	19.3 ±2.8
SCB	14 (6.3)	64.3	57.1	3±2.1	71.7±6.8	4 (28.6)	21.7±7.4
PSP	16 (7.1)	56.3	68.8	2.5±2.2	72.3±7	4 (25.0)	23.1±3.7
EA	8 (3.6)	50.0	50.0	2.0±1.7	67.5±12.5	2 (25.0)	22.7±4.3
DCL	17 (7.6)	58.8	29.4	2.4±2.1	72.8±3.8	9 (52.9)	26.8±3.3
APNF	32 (14.3)	31.3	62.5	2.4±1.5	71.4±7.8	14 (43.8)	20.7±7.7
DFTvc	99 (44.2)	54.5	70.7	3.4±2.4	69.6±10.9	51 (51.5)	22.2±6.4
DS	33 (14.7)	60.6	66.7	3.0±1.8	69.4±11.8	11 (33.3)	21.2±5.8
DFT-EMN	2 (0.9)	50.0	100	1.5±0.7	64.5±0.7	1 (50.0)	26.0±1.4
TOTAL	224	52.7	63.8	3,0±2.3	70.3±9.3	98 (43.8)	22.2±6.2

VII. Resultados

TRABAJO I

Prevalencia de mutaciones en el locus UBQLN2 en la población catalana afectada de DLFT y correlación fenotipo-genotipo de las mutaciones en UBQLN2 identificadas.

Ver Anexo: 1er artículo

TRABAJO II

Prevalencia de la expansión del locus C9ORF72 en la serie clínica e identificación del fenotipo clínico de los sujetos.

Ver Anexo: 2º artículo

Trabajo III

Identification of misdiagnosed frontotemporal dementia using APOE genotype and phenotype-genotype correlation analyses

Hernández I, Mauleón A, Rosense-Roca M, Alegret M, Vinyes G, Espinosa A, Sotolongo-Grau O, Becker JT, Valero S, Tarraga L, López OL, Ruiz A, Boada M.

Curr Alzheimer Res. 2014 Feb; 11(2):182-91.

IF: 3.676 (2014) Q1

ldentification of Misdiagnosed Fronto-Temporal Dementia Using *APOE* Genotype and Phenotype-Genotype Correlation Analyses

Isabel Hernández[1], Ana Mauleón[1], Maiteé Rosen se-Roca[1], Montserrat Alegret[1], Georgina Vinyes[1], Anna Espinosa[1], Osear Sotolongo-Grau[1], James T. Becker[2], Sergi Valero[3], Lluís Tárraga[1], Osear L. López[2], Agustín Ruiz[1,*] and Merce Boada[1,4]

[1]Fundació ACE. Barcelona Alzheimer's Treatment and Research Center, Barcelona (Spain); [2]Alzheimer's Disease Research Center. Departments of Neurology, Psychiatry and Psychology. University of Pittsburgh, Pittsburgh PA (USA); [3]Deparment of Psychiatry. Hospital Universitari Vall d'Hebron. CIBERSAM. Universitat Autónoma de Barcelona, Barcelona. (Spain); [4]Hospital Universitari Vall d'Hebron - Institut de Recerca, Universitat Autónoma de Barcelona. (VHIR-UAB), Barcelona (Spain)

Abstract: Objective: Postmortem and genetic studies of clinically diagnosed Frontotemporal dementia (FTD) patients suggest that a number of clinically diagnosed FTD patients are actually "frontal variants" of Alzheimer's disease (fvAD). The purpose of this study was to evaluare this hypothesis by combining neuropathological data, genetic association studies of APOE, phenotype-APOE genotype correlations and discriminant analysis techniques. Methods: Neuropathological information on 24 FTD cases, genetic association studies of APOE (168 FTD, 3083 controls and 2528 AD), phenotype-genotype correlations and discriminant techniques (LDA, logistic regression and decision trees) were combined to identify fvAD patients within a clinical FTD series. Results: Four of 24 FTLD patients (16.6%) met critetia for definite AD. By comparing allele and genotype frequencies of APOE in controls, FTD and AD groups and by applying the Hardy-Weinberg equilibrium law (HWE), we inferred a consistent (17.2%) degree of AD contamination in clinical FTD. A penetrance analysis for APOE ε4 genotype in the FTD series identified 14 features for discrimination analysis. These features were compared between clínical AD (n=332) and clinical FTD series (n=l68) and classifiers were constructed using linear discriminant analysis logistic regression or decision tree techniques. The classifier had 92.8% sensitivity to FTD and 93.4% sensitivity to AD relative to neuropathology (global AUC=0.939, p<<0.001). We identified 30 potential fvAD cases (17.85%) in the clinical FTD sample. Conclusion: The APOE locus association in clinical FTD might be entirely explained by the existence of "hidden" fvAD cases within an FTD sample. The degree of fvAD contamination can be inferred from APOE genotypes.

Keywords: Alzheimer's disease, apolipoprotein E4, diagnostic classification, frontotemporal lobe dementia, genetics.

INTRODUCTION

Clínical discrimination between the frontal variant of Alzheimer's disease (fvAD) and true non-AD Frontotemporal dementia (FTD) is challenging. Several studies have tried to find a useful biomarker that discriminares AD, FTD or other dementias [1 , 2]. lmpaired language and executive functions are common in both FTD and AD [3] and "frontal" behavioral symptoms may also appear in AD [4]. The clinical manifestations of frontal system dysfunction observed in some AD patients are usually correlated with the presence of neurofibrillary degeneration in the frontal lobes [5].

Patients presenting with behavioral, language and executive system abnormalities in the context of a neurodegenerative disorder are usually diagnosed with FTD. FTD comprises a group of progressive neurodegenerative disorders sub-classified according to the clínical phenotype: behavioral variant (bvFTD), semantic dementia (SD), progressive non fluent aphasia (PNF A), corticobasal syndrome (CBS), progressive supranuclear palsy (PSP), and FTD with motor neuron disease (FTD-MND).The clínical spectrum of FTD ranges from behavioral symptoms until progressive aphasic syndromes, parkinsonism plus and/or motor neuron disease. They are often associated various syndromes in the sarne subject over the clinical [6]. Although FTD and fvAD share clinical signs and symptoms, FTD is usually associated with several different histopathological features, different abnormally aggregated proteins in target neurons and the presence of a range of germline mutations in several multiple genes completely different from those found in AD [7].

Although the APOE locus is the strongest genetic risk marker for sporadic AD [8 -1 O] it is less clear whether there is a link between APOE and FTD [11 -15]. lndeed, any observed associations could be explained partially (or even totally) if the FTD clinical series was contaminated by "hidden" AD cases [16]. That is, AD cases within an FTD series would tend to inflate the E4 allele frequency, yielding an intermediare allele frequency between population-based controls and AD. The purpose of the present study was to evaluare this hypothesis [17] by combining neuropathological

* Address correspondeoce by this authors at the Fundació ACE, Marques de Sentmenat, 57, 08029 Barcelona (Spain); Tel: 34-93-444-731 8; Fax: 34-93-410- 1701 ; E-mail: aruiz@fundacioace.com

1875-5828/14 $58.00+.00

data, genetic association studies of APOE, phenotype-APOE genotype correlations and discriminant analysis teclmiques . We estimated the proportion of AD pathology in our clinical FTD series and identified potential candidate fvAD patients.

METHODS

Patients

All of the patients agreed to participate in the study and blood samples were obtained after they and/or their legal representatives provided written consent according to the GIPSY protocol approved by the ethics committee in the Hospital Clínic i Provincial (Barcelona, Spain). lnformed consent was in accordance with Spanish biomedical laws (Law 14/2007, July 3rd, about biomedical research; Royal Decree 1716/2011 , November 18th).

All patients were south-Europeans (Spanish lineage, two generation or more) recruited from the fundació ACE Bar celona Alzheimer Treatment & Research Center for APOE analysis and Hardy-Weinberg estimations data from 5779 unrelated individuals were used: 168 FTD, 2528 AD and 3083 population-based controls. The AD patients and population-based controls series have been previously described [18]. The 168 clinical FTD patients underwent a full neurological evaluation and were diagnosed either at baseline or during follow-up as clinical FTD cases by using international research criteria: bvfTD [19, 20], PNFA [21, 22], SD [23], PSP [24] and CBS [25]. Some FTD patients developed MND signs during follow-up. (Table 1-a and 1-b). Mutation screening of *MAPT* in FTD patients has been conducted in all FTD patients. We found l out of 168 canying a *MAPT* mutations (0.5% of cases). We also identified a *C90RF72* expansion in 3 out of 168 FTD (1.7%) [26]. Of note, patients canying either *MAPT* or *C90RF72* mutations were not excluded from this work. Other FTD genes have been not studied.

The Fundació ACE Memory Clinic diagnoses patients at a daily consensus conference among neurologists, neuropsychologists and social workers. Baseline signs and symptoms are acquired directly from the patient or from the primary caregiver. The patients were administered a neuropsychological battery [27] that included measures sensitive to orientation, attention, verbal learning and long-term memory, language, visuoperception, gnosis, praxis and executive functions. Neuroimaging studies were either CT and/or MRl which is required for an FTD diagnosis [20]; in sorne cases SPECT scans were also available. The locus and extent of hemispheric atrophy was rated by visual inspection by an experienced neurologist, and independently confirmed by a neuroradiologist.

Neuropathology

Postmortem neuropathology examination was performed at the Neurological Tissue Bank (NTB) of the Biobanc Hospital Clinic -IDIBAPS (Barcelona, Spain), after obtaining written informed consent from patients and/or next of kin. Brain was processed according to a standardized protocol. Histopathological evaluation was performed on multiple, formalin-fixed and parafine embedded tissue blocks.

AD-related neurofibrillary pathology was staged according to the Braak & Braak [28, 29] classification and a diagnosis of definite AD was assigned applying the CERAD criteria [30, 31] based on the semiquantitative assessment of neuritic plaque density. Both were combined using the current consensus guidelines [32].

Germline DNA Extraction and *APOE* Analysis

We extracted DNA from frozen blood using the Nucleo Spin Blood kit (Macherey-Nagel, Düren, Germany). The APOEε4 allele was identified with commercial kits for APOE rs429358 (SNP112) and rs7412 (SNP158) from Roche Diagnostics (Germany). The APOE alleles were amplified using LightCyclerApoE Mutation Detection Kit (Roche diagnostics, Germany) and detected using real-time PCR technology (LightcyclerR 480 System, Roche Diagnostics, Germany) following the manufacturer's instructions. To check the quality of the results, different compound heterozygotes for APOE SNPs were verified in an independent research laboratory. FTD-control, AD-control and HWE statistical analyses were performed manually or using online tools (http ://ihg.gsf.de/cgi-binlhw/hwal.pl).

Penetrance Analysis of *APOE*ε4 Genotype in FTD Series

We divided the FTD patients into two groups based on the presence or absence of the ε4 allele, irrespective of the allele dosage (dominant model, i.e. APOEε4 + and APOEε4 - subgroups) in order to identify the clinical, neuropsychological and neuroimaging variables (features) potentially associated with APOEε4 carrier status in FTD patients. All baseline variables were analyzed as a function of APOE genotype categories using SPSS for Windows, vl8.0 (SPSS Inc, Chicago, IL). To compare qualitative variables a standard Pearson's chi-square test was performed. To compare quantitative variables we used student's T-test or the Mann-Whitney U test (as appropriate). All of the clinical variables that showed a trend towards association ($p<0.1$; when comparing APOEε4+ and ε4- subgroups), were retained for the discriminant analyses (Supplementary Table 1).

Discriminant Analyses

To identify the linear combination of features that best discriminated between the two groups of patients with pathologically confirmed FTD or AD, an discriminant analysis was carried out [33]. Fourteen pre-selected features from the clínical FTD database were used for classifier generation (direct approach) including two qualitative neuroimaging variables (hemispheric pattern atrophy and predominant brain atrophy), presence of six clinical variables at baseline (memory deficit; behavioral symptoms; language alteration; dyslipidemia, sucking retlex and gait alteration) and six neuropsychological variables at baseline (Boston naming; verbal comprehension; semantic verbal fluency; abstract reasoning; digits span forward and Poppelreuter test). The coding of each feature, the coefficients applied to calculate the classi fier and the final discriminant function are detailed in Supplementary (Table 2).

The classifier was not biased based by the proportion of patients in each class (i.e., prior probability set to 0.5 for each class). To calculate the classifier coefficient for each variable and the constant for the canonical discriminant function, we used 332 randomly selected clinically diagnosed

Table la. Demographic characteristics of study subjects.

	Diagnostic Groups at baseline							Statisitic (p.value)[1]	D.f
	bvFTD	PNFA	SD	CBS	PSP	CI-ND*	Other*		
N(%)	74(44.0)	22(13.1)	24(14.3)	11(6.5)	11(6.5)	13(7.7)	13(7.7)		
Age at onset	66.5± 10.2	68.6±8.7	68.4± 11.6	67.6±7.5	68.3±8	70.9±4.9	67±9.2	0.513	6
Gender (%males)	43(58.1)	8(36.4)	18(75)	8(72.7)	7(58.3)	7(53.8)	5(41.7)	9.36	6
Years evolution before diagnostic (average)	3.76±2.6	2.39±1.6	2.96± 1.8	3.27±2.4	3.2±2.5	2±1	2.42±2.5	2.025	6
Family History N(%) Yes)	41(56.9)	9(40.9)	6(25)	5(45.5)	2(16.7)	9(69.2)	3(25)	17.1[2]	6
Education N(%) 6+ years	48(64.9)	15(68.2)	16(66.7)	6(54.5)	7(58.3)	4(30.8)	5(41.7)	8.25	6
$ApoE_4$ carriers frequency N(%)	22(29.7)	3(13.6)	5(20.8)	3(27.3)	2(18.2)	3(23.1)	6(46.2)	5.746	6

vbFTD: behavioral variant Frontotemporal; PNFA: Progressive non Fluent Aphasia; SD: Semantic Dementia; CBS: Corticobasal Syndrome; PSP: Progressive Suplanuclear Palsy: CI-ND: Cognitive lmpairment non dementia; Other: Unspecific Dementia at baseline.
1 - X^2 for categorical data, F for continuous data (one-way ANOVA) D.f= degree of freedom 2 - $p < .05$
*: Patient initially non classified as FTD who envolved clinical FTD during follow up.

Table 1b. Evolutive phenotype of the groups.

	Diagnostic Groups at baseline N(%)								
Evolutive phenotype N(%)	bvFTD	PNFA	SD	CBS	PSP	CI-ND	Others	Total	$ApoE_4$ carriers frequency N(%)
bvFTD	65(87.8)	1(4.5)	2(8.3)	0	0	8(61.5)	6(46.2)	82(48.8)	25(30.5)
PNFA	1(1.4)	13(59.1)	0	1 (9.1)	0	1(7.7)	1(1.4)	17(10.1)	3(17.6)
SD	2(2.7)	1(4.5)	21(87.5)	0	0	2(15.4)	0	26(1 5.5)	5(19.2)
FTD-MND	1(1.4)	1(4.5)	0	0	0	1(7.7)	0	3(1.8)	1(33.3)
CBS	1(1.4)	2(9.1)	1(4.2)	9(81.8)	0	1 (7.7)	4(30.8)	18(10.7)	5(27.8)
PSP	1(1.4)	2(9.1)	0	1(9. 1)	11(100)	0	2(15.4)	17(10.1)	3(17.6)
AD	3(4.1)	2(9.1)	0	0	0	0	0	5(3.0)	2(40.0)
Total	74(44.0)	22(13.1)	24(14.3)	11(6.5)	11(6.5)	13(7.7)	13(7.7)	168	44(26.2)

bvFTD: behavioral variant Frontotemporal; PNFA: Progressive non Fluent Aphasia; SD: Semantic Dementia; FTD-MND: frontotemporal dementia with motor neuron disease: CBS: Corticobasal Syndrome;PSP: Progressive Suplanuclear Palsy; CI-ND: Cognitive lmpairment non dementia Other: Unspecific Dementia at baseline.

Table 2. *APOE* locus genetic data available for this study.

		FTD	AD	Controls
***APOE* (Haplogenotypes)**	ε2ε2	1	5	17
	ε2ε3	9	117	317
	ε2ε4	4	41	34
	ε3ε3	114	1273	2178
	ε3ε4	37	947	508
	ε4ε4	3	145	29
	Total	168	2528	3083
***APOE* ε4 Allelic Frequency**	(%)	14%	25.3%	9.7%
***APOE* ε4 carriers**	(%)	26.2%	44.8%	18.5%
Hardy Weinberg Equilibrium (HWE)	(p-value; Pearson)	0.85	0.08	0.87

AD patients, 113 *APOE* ε4 negative FTD patients and 8 FTD patients with unknown genotype (discovery set). We used only the data from *APOE* ε4 negative patients during this initial step because, we inferred a lower frequency of fvAD cases in the FTD *APOE* ε4 negative subgroup (13%) corpored to *APOE* ε4 positive subgroup (29%). Any missing values from the features were replaced by the mean value observed in the whole series. 41 FTD *APOE* E4 positive patients with available clinical data were used to conduct a replication analysis to check the reliability of the results. Classifier scores from 332 clinical AD and 168 clinical FTD (n=500) were used to calculate the area under curve (AUC) of the proposed classifier. AUC was generated using SPSS. This exercise was conducted only to provide information about classifier performance (not for true diagnostics purposes yet). Of note, SPSS automatically calculated the best cut-off for the proposed classifier in an unsupervised manner. lndividuals with classifier values ≤-1 were classified as FTD automatically by the system. lndividuals with a classifier value >-1 were classified as AD. Fourteen FTD cases with neuropathologically confirmed disease and clinical data were used to estimate the sensitivity of the classifier to detect true FTD individuals.

Sensitivity Analysis

Other discriminant techniques were used to demonstrate the existence of "AD information" within FTD series. Specifically, to further reassure that results were not technical artifacts inherent to LDA method limitations, we conducted a sensitivity analysis by using two additional statistical approaches. Briefly, the prognostic utility of selected variables was investigated by an unsupervised decision tree approach entering all selected variables. Two variables were automatically selected by the system: node 0: "predominant brain atrophy" divided in three sub-nodes. Node 1: temporal pole or fronto-temporal and parietal atrophy or unknown. Node 2: parietal atrophy (with or without language alterations; node 4 or 5) and node 3: temporal-parietal or hippocampal atrophy. Of note, training and validation samples were identical to those used in LDA. Sensitivity (fraction FTD) and specificity (fraction EA) values were produced. Data were considered significant when the P-value was below 0.05. Concordance between LDA and decision trees was calculated by using a simple 2x2 chi-square test among classified subjects. Concordant classification was obtained for 86.8% of individuals ($p=3.47x10^{-55}$) (Supplementary Table 5).

Finally, a binary logistic regression approach was conducted by using identical 14 features entered during discriminant analysis or decision tree experiments. By comparing predicted phenotypes by LDA or logistic regression, an identical classification of individuals was obtained for 92.4% of individuals ($p=7.87x10^{-48}$). Concordance between decision tree classification and logistic regression was also measured (97.1%; $p=7.83x10^{-67}$)(Supplementary Table 5).

Phenotype Genotype Correlations

The classifier algorithm identified 30 FTD patients (J 7 .85%) that had classifier scores in the range of AD (i.e.,score >-1). So, we conducted a new exhaustive phenotype analysis including demographic, clinical, neuropsychological and follow-up data of the FTD series by dividing it in two groups (genuine "FTD" and fvAD) .

RESULTS

Histopathological examination of the 24 FTD patients revealed that 4 (16.6%) met criteria for Definite AD without any other pathological evidence or protein deposit related to an FTD phenotype. A summary of each FTD patient with inconsistent clinical and pathological findings is provided in Supplementary Table 3. The remaining cases were classified pathologically as FTLD-TDP (N=ll(45.8%)], FTLD-TAU (N= 8 (33.3%)] and FTLD-FUS (N= 1 (4.1 %)].

We observed the expected E4 allele differences when comparing AD and controls (44.8% vs. 18.5% respectively; APOE E4 allele Odds Ratio= 3.2; 95% Confidence Interval (C.L) = [2.839-3.511); p<<.001). To calculate potential AD contamination in FTD series, we compared the E4 allele frequencies between FTD, AD and the population-based controls (Table 2). Two assumptions were made: a) the E4 allele frequency does not deviate from the HWE law expectation and b) any increase of E4 allele frequency in FTD would be due to contamination by AD cases. There was a significant "excess" of E4 carriers in the FTD series when compared with population based controls (26.2% vs. 18.5% carriers; Allele Odds Ratio= l.5 (95% Cl = (1.104-2.093]), p=.009). The observed allele frequency inflation in the clinical FTD cases (i .e., 26.2 - 18.5 = 7.7%) was used to deduce the fraction of fvAD individuals not carrying this allele. Taking into account that 44.8% of AD individuals were E4+ (Table 2), we inferred the fraction of fvAD non-carriers contaminating the FTD series by using a simple model of three (assuming HWE) which results in 9.5% of the FTD series. The global estimation of "hidden" AD cases within our clinical FTD series was obtained by summing the excess of E4 carriers observed and the corresponding estimate of noncarriers assuming HWE (i.e., 7.7+9.5= 17.2%). The genetics based estimation suggests that about 29 FTD cases may, in fact, be fvAD patients. This result is not statistically different from that observed in the neuropathological series (16.6%) (p=.69; Fisher's exact test).

A penetrance analysis was conducted to identify phenotype differences between *APOE*ε4+/- FTD subgroups (Supplementary Table 1). Fourteen variables (features) had moderate association (p< .1) with genotype (Table 3). A numeric classifier using selected features was constructed using discriminant analysis techniques and was applied to 500 individuals (332 clinical AD vs 168 clinical FTD; 14 of these with pathological confirmation). The classifier resulted in 91.9% sensitivity (90.7% cross-validation) to AD (global AUC= .939, p<<.001), and correctly classified 92.8% of the postmortem FTD cases (13/14 pathological FTLD with whole phenotype available for discriminant analysis). Similar results were obtained by using decision tree or binary Jogistic regression approaches (supplementary Table 5)

Of note, the classifier displayed a consistent, lower sensitivity for FTD c.4 carriers (82.7%) and non-carriers (80.5%) compared to AD (both groups were separately analyzed) (supplementary Table 5). Thirty FTD patients (17.85%) were assigned as fvAD, which is consistent with our previous

Table 3. Candidates discriminant variables derived from penetrance analysis.

Variable	APOE Status		Statistics	
	E4-	E4+	χ^2	t
Hemisfcric Pattern atrophy (% (N))				
Lelf	34.7 (35)	8.8 (3)	0.013	
Right	10.9 (11)	11.8 (1)		
Symmetric	54.5 (55)	79.4 (27)		
Predominant Brain atrophy (%(N))				
Frontal	17.8 (18)	32.4 (11)	0.082	
Temporal	31.7 (32)	20.6 (7)		
Parietal	3.0 (3)	11.8 (4)		
Fronto-temporal	30.7 (31)	23.5 (8)		
Fronto-parietal	3.0 (3)	5.9 (2)		
Global	13.9 (14)	5.9 (2)		
lnitial symptom reported by caregivcr (%(N))				
Memory	35 (41)	50 (20)	0.094	
Behavior	41.4(46)	64.1 (25)	0.015	
Language	53.2 (59)	33.3 (13)	0.033	
Pathological background (% (N))				
Dyslipemia	32.8 (38)	47.5 (19)	0.095	
Neurological exploration (%(N))				
Sucking	1 (1)	5.7 (2)	0.092	
Normal gait	66.4 (75)	81.6 (31)	0.076	
Altered gait	33.6 (38)	18.4 (7)		
Ncuropsychological exploration. Quantitative evaluation (average)				
Visual Naming (15-BNT)	10.42±3.96	12.09±3.34		0.036
Verbal comprehemsion	4.91 ± 1.3	5.53±0.96		0.006
Semantic verbal fluency	8.48±4.55	11.03±3.94		0.005
Similarities WAlS-III	5.49±3.50	7.34±3.60		0.016
Neuropsychological exploration. Qualitative evaluation impaired (% (N))				
Poppelreuter test	48.2 (41)	2!.2 (7)	0.007	
Digit Span Forward WAlS-III	72.2 (65)	54.5 (18)	0.064	

χ^2 = Pearson Chi-square
t= Levene for equality of variances

estimation from the pathological series (16.6%; p= .64) and HWE methods (17.2%; p= .88) which also predicted a similar number offvAD cases (n=29).

In order to identify clinical differences, the series was split based on the results of the classifier (fvAD versus genuine FTD) (Table 4). The FTD patients who were classified as fvAD had a higher frequency of change of diagnosis during the course of the disease (31% versus 16.9%; p=.Ol). In addition, there were statistically significant differences between groups in terms of the presence of memory complaints or absence of behavioral symptoms at baseline (Table 4). The MMSE score did not vary between the two groups (ANOYA, eta2= .001. p>.05), nor did multiple neurological and neuropsychological variables differ. Neuroimaging data suggested that differences in predominance of hemispheric atrophy for each of the groups might be useful for selecting fv AD candidates.

Given that FTD is more common in presenile age (especially the behavioral variant phenotype), we also separated the clinical series (N = 168) into older and younger than

Table 4. Characteristics of patients as a function of diagnosis and classifier algorithm.

	Diagnosed FTD			**Diagnosed AD (AD)**		
	Classified FTD (FTD)	**Classified AD (fvAD)**	**Effect Size** (FTD vsfvAD)		**Effect Size (p-value)** (AD versus fvAD)	**Effect Size (p-value)** (AD versus FfD)
Demographics						
N	138	30	Na	332	Na	Na
Age	70.6±9.8	72.8±7.3	Eta^2=0.005(0.3)	81.4±7.6	Eta^2=0.11(p<0.001)	Eta^2=0.29(p<0.001)
Education (% <6years)	37%	46%	OR= l .4(0.3)	48%	OR=0.94(0.8)	OR=0.63(0.02)
Sex (male %)	55.8%	60%	OR= 1.18(0.68)	25%	OR=4.3 (<0.001)	OR=3.6 (<0.001)
Mini-Mental State Score	22.2±6.8	22.6±6.2	Eta^2=0.001(0.7)	19.2±3.9	Eta^2=0.04(<0.001)	Eta^2=0.067(<0.001)
Family History (% Yes)	37.2%	57.1%	OR=2.25(0.06)	46.6%	OR= l.5(0.28)	OR=0.68(0.08)
ApoE4 carriers frequency N(%)	35(25.4%)	9(30%)	Na			
Phenotype						
Chief complaints at baseline						
Focal Signs	10%	3%	P(6dt)= l.7E-5	0%	P(4dt)=0.01	P(6d t)= 3E-34
Behavior	19.6%	0%		1.5%		
Gait disturbance	1.4%	0		0%		
Memory	34.1%	90%		89.2%		
Language	1.4%	0		0		
Other	33.5%	7%		9.3%		
Symptoms reported by the caregiver at the beginning of the process						
Depression	8.7%	0	P(8dt)=6.8E-12	1.5%	P(6dt)=0.8	P(8dt)=9.5E-47
Job Loss	0.7%	0		0		
Focal Signs	15.2%	0		0.6%		
Behavior	29.8%	0		3.3%		
Gait disturbance	2.2%	0		0.6%		
Memory	20%	100%		90.7%		
Language	1.4%	0		0		
Others	22%	0		3.3%		
Predominant signs at baseline						
Agitation	5.0%	0%	OR=0.80(0.59)	2.1%	OR=0.92(1)	OR=2.4(0.12)
Bulimia	7.5%	0%	OR=0.80(0.21)	0%	Na	OR=0.25(<0.001)
Depression	24.2%	0%	OR=0.77(0.02)	24.5%	OR=0.91 (0.001)	OR=0.984(1.0)
Disorientation	26.7%	29.6%	OR= 1.15(0.81)	56%	OR=0.3 3(0.009)	OR=0.28(<0.001)
Hypersexuality	4.2%	0%	OR=0.81(0.58)	0%	Na	OR=0.26(0.001)
Delusions	10%	11.1%	OR= l. l (l)	10.1%	OR= l.l (0.74)	OR=0.99(1)
lnhibition	6.7%	0%	OR=0.8(0.35)	1.5%	OR=0.92(1)	OR=4.6(0.008)
Memory loss	71.7%	96.3%	OR=10.2(0.005)	98.5%	OR=0.40(0.381)	OR=0.39(<0.001)

(Table 4) contd...

	Diagnosed FTD			Diagnosed AD (AD)		
	Classified FTD (FTD)	**Classified AD (fvAD)**	**Effect Size** (FTD vsfvAD)		**Effect Size (p-value)** (AD versus fvAD)	**Effect Size (p-value)** (AD versus FfD)
Focal sings	8.3%	3.7%	OR=0.42(0.69)	0.3%	OR= 12.5(0.14)	OR=29.6(<0.001)
Gait disorder	10.8%	0%	OR=0.79(0.12)	3.7%	OR=0.92(0.61)	OR=3.1(0.009)
Language disorder	46.7%	33.3%	OR=0.57(0.28)	25.1%	OR= 1.4(0.36)	OR=2.6(<0.001)
Neuroimaging data						
Symmetry Pattern						
Rt. Hemjsphere	14.3%	4.8%	P(2dt)=0.002	1.5	P(2dt)=0.15	P(2dt)=5.7E-14
Lt. Hemisphere	31.3%	0%		11.2		
Symmetric	54.5%	95.2%		87.3		
Atrophy Location						
Frontal	26.8%	0	P(5dt)=5.8E-6	0.6%	P(7dt)=4.5E-29	P(7dt)=4.24E-64
Temporal Pole	34.8%	0		1.2%		
Parietal	4.5%	4.8%		2.4%		
FrontoTemporal	21.4%	66.7%		2.1%		
Global	8.9%	28.6%		49.5%		
Fronto Parietal	3.6%	0		0.3%		
Hippocampus	0	0		32.3%		
TemporoParietal	0	0		11.5%		

Note: p- values appear in bracket or alone on each cell. P-values below 0.0011 are declared significant (Bonferroni's Correction for multiple comparisons; 43 tests). Df=degree of freedom. Na=Nor applicable.

65 years. We analyzed the effect of age at onset of symptoms on the clinical variables, regardless of ApoE4 genotype. (Supplementary Table 4)

The bvFTD phenotype was more often observed in those below 65 years. Patients below 65 years had a higher percentage of family history of dementia (55.8% p= .028). Younger patients showed more behavioral alteration at the onset of symptoms (64.1% p= .005). Besides, memory impairment was the most frequent symptom in the older ones (46.3% p= .012). Moreover the pathological background showed that dyslipidemia (42.3%, p= .012), heart disease (21.3%, p = .031) and osteoarthrosis (29.4%, p<.001) were more frequent in the older population and this is not surprising taking into account that this clinical findings increases with age. There were no further significant differences on neurological variables. Regarding the neuropsychological variables, only the SKT test and the clock test were significant in the older group. Of note, neither the neurological examination variables nor the neuroimaging variables discriminated by age at symptoms onset.

DISCUSSION

There are three main findings from the present study. First, based on neuropathological analysis, approximately 17% of the clinically diagnosed FTD patients met neuropathological criteria for definite AD. Second, it was possible to create a classifier algorithm using both genotype and phenotype information to identify those clinical FTD patients who were actually more likely to be AD. Third, when comparing the variables of these "hidden" AD cases within the FTD sample, they were more likely to be classified as clinical FTD than fvAD.

Although the problem of "hidden" AD in clinical series of FTD patients is well known, this is the first time that it has been possible to create a classifier that was able to identify individuals within a sample of clinical FTD who were at very high risk for having AD. Small studies have been inconsistent in terms of finding an association between APOE and FTD [11, 12, 13, 14, 34-40] although neuropathological series generally fail to demonstrate any link between the ε4 allele and FTD [41]. In addition, a recent GWAS using exclusively neuropathological TDP-43+ (FTLD-TDP) cases [11, 42] failed to isolate any significant signal around the APOE locus. Thus, our data are fully consistent with the idea that the E4 allele is not associated with FTD risk, and any apparent increases in allele frequency are entirely due to misdiagnosed AD cases within the clinical FTD series. However, last interpretation has sorne limitations. For example, there are not GWAS data for FTLD-TAU or FTLDFUS. Further research is needed in order to guarantee that

any apparent increases in allele frequency are entirely due to misdiagnosed AD cases within the clinical FTD series.

Clinicians and researchers must be cautioned that despite the "hidden" AD cases within the group with FTD, it would not be appropriate to eliminate patients with a ε4 allele from either clinical series or research studies of FTD. To do so would remove approximately two-thirds of the genuine FTD cases. Therefore, the most fruitful approach would be to use a statistical classifier to identify those individuals within the FTD sample who were most likely to be "hidden" AD cases, and to treat them as a separate group for analysis, as shown here. While this does not completely eliminate the problem of the contamination of the FTD series, it may help to minimize the impact of that contamination on the outcomes of interest.

The identification of individuals who were diagnosed with FTD but had AD is important since the misdiagnosis can result in the failure to provide appropriate medication for the patient, or even potentially the prescription of inappropriate medications [43]. The behavioral phenotype, alone, is not sufficient to disentangle these two clinical syndromes, but using both genetic and neuro-imaging markers along with the clinical signs/symptoms, has the potential to improve patient classification and thus case management. Indeed, it may be that among patients diagnosed with FTD who are ε4 carriers, it would be important for them to be able to have more specific neuroimaging assessment, using either FDG metabolic scans or in vivo amyloid imaging to help to clarify the diagnosis.

It is vital to emphasize the need for a reliable estimate of ε4 allele frequency; a large series of AD and controls (e.g., n>1000) is an essential component of this kind of research. We used estimates based on more than eleven thousand chromosomes (5611 individuals) to calculate fvAD contamination. The precision of the e4 frequencies facilitated the inference of fvAD (17.2%) that resulted in almost identical rates compared to the postmortem data (16.6%).

The penetrance and discriminant analyses were used to indirectly demonstrate that the information contained in clinical variables differentiating *APOE*ε4 positive and negative FTD patients may also differentiate AD cases and genuine FTD (postmortem) with relatively good precision (AUC 93.9%).

However, the proposed classifier needs independent replication and could be improved by extending the discriminant analysis to the whole clinical database and by including age at onset and *APOE* genotype. Of note, *APOE* was only used in the first phase of the study (penetrance analysis), but not in the discriminant analysis which led to the score. In addition the results of the discrirninant average scores do not vary with the age of onset of the disease. So, we feel that by adding *APOE* or age at onset to the discriminant analysis we cannot improve discrimination too much. Further research increasing sample size and using deep phenotyping would be necessary to improve obtained discriminant function.

Although AD and FTD can be differentiated using histopathological analysis the existence of the fv AD complicates the clinical identification of genuine FTD patients. From a clinical point of view, the best predictor to identify hidden fvAD in FTD series is memory impairment (Table 4). Moreover, behavior changes in the genuine FTDs such apathy, desinhibition, or eating changes, are the first complaints referred by relatives. Besides, fvAD and FTD might have different neuroimaging atrophy patterns (Table 4).

The characterization of the phenotype of rare FTD syndromes is difficult and may affect the identification of valid genetic markers for FTD using GWAS strategies and other massive molecular techniques. Most important, the misclassification of AD patients as FTD might hamper the access to palliative or empírical therapies by these atypical AD cases. Consequently, the improvement of mathematical tools to comprehensively differentiate fvAD and FTD would be of interest not only for research but also for clinical purposes in the future.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

ACKNOWLEDGEMENTS

This work was carried out as part of the doctoral program of I. Hernández at the Autonomous University of Barcelona. This work was partially supported by the Spanish Ministry of Health from Instituto de Salud Carlos III (Madrid) (FISS PI10/00945) and by the Agència d'Avaluació de Tecnologia i Recerca Mèdiques. Departament de Salut de la Generalitat de Catalunya (Health Department of the Catalan Government) (390) and by the University of Pittsburgh Alzheirner's Disease Research Center (AG05133). We are indebted to Trinitat Port-Carbó and her family for their support of the fundació ACE research programs.

We thank brain donors and their families for generous brain donation for research. The authors are indebted to Dr. Maria Jesus Rey and Dr. Ellen Gelpi from the Neurological Tissue Bank of the Biobank-Hospital Clinic -IDJBAPS for detailed neuropathological brain examination.

SUPPLEMENTARY MATERIAL

Supplementary material is available on the publishers web site along with the published article.

STUDY FUNDING

FJS/EC11 -358 and FIS/P: 10-00945 of Ministerio de Sanidad, Servicios Sociales e Igualdad. Spain; AATM/390-6-2009. Generalitat de Catalunya (Catalan Government) NIA/NIH (AG05133).

AUTHOR DISCLOSURES

Isabel Hernández has nothing to disclose.

Ana Mauleón has nothing to disclose.

Maiteé Rosense-Roca has nothing to disclose.

Montserrat Alegret has nothing to disclose.

Georgina Vinyes has nothing to disclose.

Ana Espinosa has nothing to disclose.

Sergi Valero has nothing to disclose.

Osear Sotolongo-Grau has nothing to disclose.

James T. Becker is supported, in part, by funds from the NIH (AG05133, AG034852, AG020098, MH098745, A1035041).

Lluís Tárraga has nothing to disclose.

Osear L. López has served as a consultant to Lunbeck and Mertz pharmaceuticals. He is supported, in part, by funds from the NIH (AG05133, AG020098, AG024904, MH09033, AG022376, AG037451, AG025204, HD055525).

Agustín Ruiz is a shareholder of Neopharm Obesity SL and Oxigeneinc.

Merce Boada is consultant to Novartis and Esteve pharmaceuticals. She is supported, in part by funds from FIS/ECll-358 and FISIP: 10-00945 of Ministerio de Sanidad, Servicios Sociales e Igualdad. Spain ; AATM/390-6-2009. Generalitat de Catalunya (Catalan Government). She is a member of Advisory Boards of Grifols, Lilly, Elan, Nutricia, Genentec and Roche.

ABBREVIATIONS

FTD	=	Clinical Fronto-temporal dementia
fvAD	=	Clinical "frontal variant" Alzheimer's disease
AD	=	Alzheimer's Disease
FTLD	=	Histological Fronto-temporal Lobar de generation
bvFTD	=	Behavioral variant Fronto-temporal de mentia
PNFA	=	Progressive Non Fluent Aphasia
SD	=	Semantic Dementia
PSP	=	Progressive Supranuclear Palsy
CBS	=	Cortico Basal Syndrome
FTD-MND	=	Fronto-temporal Dementia with Motor Neuron Disease
APOE	=	Apolipoprotein E genotype
HWE	=	Hardy-Weinberg equilibrium law
LDA	=	Linear discriminant analyses
FTLD-TAU	=	Fronto-temporal Lobar degeneration with TAU inclusions
FTLD-TDP	=	Fronto-temporal Lobar degeneration with TDP-43 inclusions
FTLD-FUS	=	Fronto-temporal Lobar degeneration with FUS inclusions.

REFERENCES

[1] Clerx L, Jacobs HL, Burgmans S, Gronenschild EB, Uylings H, Echávarri C, et al. Sensitivity of Different MRl-Techniques to Assess Gray Matter Atrophy Pattems in Alzheimer's Disease is Region-Specific. Curr. Alzheimer Res 1 0(9): 940-51 (2013).

[2] Mukaetova-Ladinska EB, Abdeii-AII Z, Andrade J, da Silva JA, Boksba 1, Burbaeva G, el al. Platelet Tau Protein as a Potential Peripberal Biomarker in Alzheimer's disease: An Explorative Study. Curr. Alzheimer Res 1 0(9): 987- 1004 (2013).

[3] Becker JT, Huff FJ, Nebes RD, Holland A, Boller F. Neuropsycbological function in Alzheimer's disease. Pattem of impairment and rates of progression. Arch Neurol 45(3): 263-8 (1988).

[4] Stern Y, Mayeux R, Sano M, Hauser WA, Bush T. Predictors of disease course in patients with probable Alzheimer's disease. Neurology 37(10): 1649-53 (1987).

[5] Jobnson JK, Head E, Kim R, Starr A, Cotman CW. Clínical and pathological evidence for a frontal variant of Alzheimer disease. Arch Neurol56(10): 1233-9 (1999).

[6] Kertesz A, McMonagle P, Blair M, Davidson W, Munoz DG. The evolution and patbology of frontotemporal dementia. Brain 128(Pt9): 1996-2005 (2005).

[7] Goedert M, Ghetti B, Spillantini MG. Frontotemporal dementia: implications for understanding Alzheimer disease. Cold Spring Harb Perspect Med 2(2): a006254 (20 12).

[8] Corder EH, Saunders AM, Strittmatter WJ, Schmechel DE, Gaskell PC, Small GW, *el al.* Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. Science 261 (5123): 921 -3 (1993).

[9] Lovati C, Galimberti D, Albaní D, Bertora P, Venturelli E, Cislaghi G, et al. APOE ε2 and ε4 influence the susceptibility for Alzheimer's disease but not other dementias. lnt. J. Mol Epidemial Genet 1 (3): 193-200.

[10] Hauser PS, Ryan RO. lmpact of Apolipoprotein E on Alzheimer's Disease. Curr Alzheimer Res 10(8):809-17 (2013).

[11] Seripa D, Bizzarro A, Panza F, Acciarri A, Pellegrini F, Pilotto A, *et al.* The APOE gene locus in frontotemporal dementia and primary progressive aphasia. Arch Neurol Arch Neurol 68(5): 622-8 (2011).

[12] Acciarri A, Masullo. C, Bizzarro A, Valenza A, Quaranta D, Marra C, el al. Apoe epsilon2-epsilon4 genotype is a possible risk factor for primary progressive aphasia. Ann. Neurol 59(2): 436-7 (2006).

[13] Stevens M, van Duijn CM, de Knijff P, van Broeckhoven C, Heutink P, Oostra BA, el al. Apolipoprotein E gene and sporadic frontal lobe dementia. Neurology 48(6): 1526-9 (1997).

[14] Gescbwind D, Karrim J, Nelson SF, Miller B. The apolipoprotein E epsilon4 allele is not a significant risk factor for frontotemporal dementia. Ann. Neurol Neurobiol Aging pii: SO 197-4580(13)00614-3 (2013).

[15] Bigio EH, Mishra M, Hatanpaa KJ, White CL, Jobnson N, Rademaker A, et al. TDP-43 pathology in primary progressive aphasia and frontotemporal dementia with pathologic Alzheimer disease. Acta Neuropatbol 120(1): 43-54 (2010).

[16] Liscic RM, Storandt M, Caims NJ, Morris JC. Clínical and psychometric distinction of frontotemporal and Alzheimer dementias. Arch Neurol Arcb Neurol 64(4): 535-40 (2007).

[17] Zintl M, Petkov M, Scbmitz G, Hajak G, Klünemann H-H. Frontotemporal dementia in association with a family history of dementia and ApoE polymorphism. Nervenarzt 81 (1): 75-8 (2010).

[18] Sesbadri S, Fitzpatrick AL, llera m MA, DeStefano AL, Gudnason V, Boada M, et al. Genome-wide analysis of genetic loci associated with Alzbeimer disease. JAMA 303(18): 1832-40 (2010).

[19] Neary D, Snowden JS, Gustafson L, Passant U, Stuss D, Black S, *et al*. Frontotemporal lobar degeneration: a consensus on clinical diagnostic criteria. Neurology 51 (6): 1546-54 (1998).

[20] Rascovsky K, Hodges JR, Knopman D, Mendez MF, Kramer JH, Neubaus J, *et al.* Sensitivity of revised diagnostic criteria for the behavioural variant of frontotemporal dementia. Brain 134(Pt 9):2456-77 (20 11).

[21] Mesulam MM. Slowly progressive apbasia witbout generalized dementia. Ann Neurol 11 (6): 592-8 (1982).

[22] Gomo-Tempini ML, Hillis AE, Weintraub S, Kertesz A, Mendez M, Cappa SF, *et al*. Classification of primary progressive aphasia and its variants. Neurology 76(11): 1006-14 (2011).

[23] Hodges JR, Patterson K, Oxbury S, Funnell E. Semantic dementia. Progressive fluent apbasia with temporal lobe atrophy. Brain [Internet]. 1992 Dec [cited 2012 Dec 14];115 (Pt 6:1783- 806. Available from: http://www.ncbi .nlm.nih .gov/pubmed/1486461

[24] Steele Jc, Richardson Jc, Olszewski J. Progressive Supranuclear Palsy. A beterogeneous degeneration involving the Brain Stem, Basal Ganglia and Cerebellum with Vertical Gaze and Pseudobulbar Palsy, Nucbal Dystonia and Dementia. Arch Neurol 10: 333-59 (1964).

[25] Boeve BF, Lang AE, Litvan L corticobasal degeneration and its relationship to progressive supranuclear palsy and frontotemporal dementia. Ann Neurol 54 (5): S 15-9.

[26] Xi Z, Zinman L, Grinberg Y, Moreno D, Sato e, Bilbao JM, *et al.* lnvestigation of e9orf72 in 4 Neurodegenerative Disorders. Arch Neurol 2012 l -8 (2012).

[27] Alegret M, Espinosa A, Yinyes-Junqué G, V alero S, Hernández I, Tárraga L, et al. Normative data of a brief neuropsychological battery for Spanish individuals older than 49. J elin Exp Neuropsychol l 34(2): 209-.19 (20 12).

[28] Braak H, Braak E. Neuropathological stageing of Alzheirnerrelated changes. Acta Neuropathol82(4): 239-59 (1991).

[29] Braak H, Alafuzotf 1, Arzberger T, Kretzschmar H, Del Tredici K. Staging of Alzheimer disease associated neurofibrillary pathology using paraffin sections and immunocytochemistry. Acta Neuropatholll2(4): 389-404 (2006).

[30] Morris Je, Heymao A, Mohs Re, Hughes JP, van Belle G, Fillenbaum G, et al. The consortium to Establish a Registry for Alzheimer's Disease (eERAD). Part I. clinical and neuropsychological assessment of Alzheimer's disease. Neurology; 39(9): 1159-65 (1989).

[31] Mirra SS, Heyman A, McKeel D, Sumi SM, erain BJ, Brownlee LM, el al. The consortium to Establish a Registry for Alzheimer's Disease (eERAD). Part Ll . Standardization of the neuropathologic assessment of Alzheimer's disease. Neurology 41 (4): 479-86 (1991).

[32] Montine TJ, Phelps eH, Beach TG, Bigio EH, eaims NJ, Dickson DW, *et al.* National lnstitute on Aging-Alzbeimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease: a practica [approach. Acta Neuropathol 123 (1): 1- 11 (2012).

[33] Wolz R, Julkunen Y, Koikkalainen J, Niskanen E, Zhang DP, Rueckert D, et al. Multi-method analysis of MRJ images in early diagnostics of Alzbeimer's disease. PLoS One 6(1 0): e25446 (2011).

[34] Gustafson L, Abrabamson M, Grubb A, Nilsson K, Fex G. Apolipoprotein-E genotyping 111 Alzheimer's disease and frontotemporal dementia. Dement GeriatrCogn Disord 8(4): 240-3 (1997).

[35] Pickering-Brown SM, Siddons M, Mann DM, Owen F, Neary D, Snowden JS. Apolipoprotein E allelic frequencies in patients with lobar atrophy. NeurosciLett 1995 188(3): 205-7 (1995).

[36] Masullo e , Daniele A, Fazio YM, Seripa D, Gravina C , Filippini Y, *et al*. The Apolipoprotein E genotype in patients affected by syndromes with focal cortical atrophy. Neurosci Lett 303(2): 87-90 (2001).

[37] Lehmann DJ, Smith AD, eombrinck M, Bametson L, Joachim C. Apolipoprotein E epsilon2 may be a risk factor for sporadic frontotemporal dementia. J Neurol Neurosurg Psychiatry 69(3): 404-5 (2000).

[38] Yerpillat P, Camuzat A, Hannequin D, Thomas-Anterion C, Puel M, Belliard S, el al. Apolipoprotein E gene in frontotemporal dementia: an association study and meta-analysis. Eur J Hum Genet 10(7): 399-405 (2002).

[39] Minthon L, Hesse C, Sjogren M, Englund E, Gustafson L, Blennow K. The apolipoprotein E epsilon4 allele frequency is normal in fronto-temporal dementia, but correlates with age at onset of disease. Neurosci Lett 226(1): 65-7 (1997).

[40] Daniele A, Matera MG, Seripa D, Acciarri A, Bizzarro A, Pilotto A, el al. APOE epsilon 2/epsilon 4 genotype a risk factor for primary progressive aphasia in women. Arch Neurol 66(7): 910--2 (2009).

[41] Nielsen AS, Ravid R, Kampborst W, Jergensen OS. Apolipoprotein E epsilon 4 in an autopsy series of various dementing disorders. J Alzheirners Dis 2003 5(2): 119-25 (2003).

[42] Van Deerlin YM, Sleiman PMA, Martinez-Lage M, Cben-Plotkin A, Wang L-S, Gratf-Radford NR, el al. Common variants at 7p21 are associated with frontotemporal lobar degeneration with TDP-43 inclusions. Nat Genet 42(3): 234-9 (20 1 0).

[43] Grossman M, Onyike C, Graf-Radford N, Mendez M, Kerwin D, Lemer A, el al. Memantine in patients with frontotemporal lobar degeneration: a multicentre, randomised, double-blind, placebo-controlled trial. Lancet Neurol 12(2): 149-56 (20 13).

Received: July 03,2013 **Revised: November 04, 2013** **Accepted: November 08, 2013**

Trabajo IV

Association of TMEM106B rs1990622 marker and Frontotemporal dementia: evidence for a recessive effect and meta-analysis.

Hernández I, Rosense-Roca M, Alegret M, Mauleón A, Espinosa A, Vargas L, Sotolongo-Grau O, Tarraga L, Boada M, Ruiz A. Journal Alzheimer's Disease.

Volume 43, Number 1, IN PRESS

IF: 4,17 (2014) Q2

Journal of Al zheimer's Disease (2014)
DOI 1 0.3233/JAD- 132432
JOS Press

Association of *TMEM106B* rs 1990622 Marker and Frontotemporal Dementia: Evidence for a Recessive Effect and Meta-Analysis

Isabel Hernández, Maitée Rosende-Roca, Montserrat Alegret, Ana Mauleón, Ana Espinosa, Liliana Vargas, Oscar Sotolongo-Grau, Lluís Tárraga, Mercè Boada and Agustín Ruiz.*

Alzheimer Research Center and Memory Clinic. Fundació ACE. Institut Català de Neurociències Aplicades. Barcelona, Spain.

Handling Associate Editor: Beoedetta Nacmias

tAccepted 3 June 2014

Abstract. Transmembrane Protein 106B SNP rs1990622 was recently shown to modify the risk of Frontotemporal Lobar Degeneration with TDP-43 inclusions (FTD-TDP). An independent replication study of this genetic variant was performed in 381 individuals from Catalonia (Spain). By applying a recessive model, a tendency towards an association with FTD risk was observed in our case–control study (age and sex-adjusted odds ratio=0.57; p=0.082). Importantly, meta-analysis of available studies also supports a recessive effect for rs1990622 CC genotype (OR=0.70; C.I. 95% [0.57-0.85]; p=0.0003) and demonstrates the existence of statistical heterogeneity due to an inherent pathological heterogeneity between series (P=0.00014). We conclude that TMEM106B is associated with FTD, although the extent of this effect is difficult to be estimated by using clinical FTD series.

Key Words: Frontotemporal Dementia, Genetics, GWAS, TMEM106B, molecular epidemiology

INTRODUCTION

Frontotemporal Lobar Degeneration (FTLD) is a fatal neurodegenerative disorder in adults. In fact, FTLD is the second cause of dementia, after early onset Alzheimer's disease (EOAD), in people under 65 years old. From a clinical point of view, FTD (Frontotemporal dementia) is a commonly used term. FTD comprises several disorders with very specific clinical hallmarks. The most common FTD subtype is the behavior variant (bvFTD), characterized by behavioral and personality changes such as disinhibition, that often coexists with apathy and manifests itself by impulsive behavior, loss of empathy, or stereotypic behavior, leading to a loss of social competence [1] an international consortium developed revised guidelines for the diagnosis of behavioural variant frontotemporal dementia. The validation process retrospectively reviewed clinical records and compared the sensitivity of proposed and earlier criteria in a multi-site sample of patients with pathologically verified frontotemporal lobar degeneration. According to the revised criteria, 'possible' behavioural variant frontotemporal dementia requires three of six clinically discriminating features (disinhibition, apathy/inertia, loss of sympathy/empathy, perseverative/compulsive behaviours, hyperorality and dysexecutive neuropsychological profile. Executive functions are impaired and in the initial stages of the disease, memory and visuo-perceptive skills are well preserved. In contrast, Progressive Non Fluent Aphasia (PNFA) or Semantic Dementia (SD) are characterized by language disturbances including impaired expressive language, difficulties naming objects and reduction of verbal fluency or loss of comprehension of word meaning respectively [2]. FTLD may be associated with Motoneuron Disease (MND), in particular Amiotrophic Lateral Sclerosis (ALS), or with parkinsonism. Several authors recognize clinical, genetic and pathological overlap between FTLD and ALS [3-5].

The clinical FTD phenotype is heterogeneous, but pathological findings in postmortem analyses of brains from subjects diagnosed of clinical FTD have also

***Correspondence to:** Agustín Ruiz, M.D. PhD, Fundació ACE, Marquès de Sentmenat, 57 08029 Barcelona (Spain). Tel. 34-93-444-7318; Fax.34-93-410-1701; E-mail: aruiz@fundacioace.com

revealed at least three different immunohystochemical phenotypes related to specific protein deposits. Around 40% of patients with clinical FTD show tau inclusions. They comprise most cases of PNFA, ~45% of cases of bvFTD and some cases of SD [6-7]. More than 50% of the FTLD patients present tau-negative ubiquitin staining inclusions referred to as FTLD-ubiquitin (FTLD-U) and in 80-90% of this group inclusions are composed of transactive response (TAR) DNA-binding protein 43 [8] small numbers of cases have recently been reported with TDP-43-negative FTLD-U pathology. To determine the frequency and to define the clinico-pathological spectrum of TDP-43-negative FTLD-U, we re-evaluated 44 cases with a previous diagnosis of FTLD-U or dementia lacking distinctive histopathology. We identified nine cases (20%. However 10% of these cases are composed of a heterogenous collection of uncommon disorders. These cases with FTLD-U show no evidence of abnormal TDP-43 and several tau/TDP-43-negative FTLD subtypes are immunoreactive (ir) for the fused in sarcoma (FUS) protein [9]. Cases with inclusions that can only be demonstrated by immunohistochemistry against proteins of the ubiquitin proteasome system (UPS), are designated FTLD-UPS [10]. FTD-3 (mutations in the *CHMP2B* gene) is the major entity associated with this FTLD-UPS pathology. This designation recognizes that the TDP-43-negative inclusions may immunostain for UPS proteins other than ubiquitin, such as p62. Recently, unconventional non-ATG translation of the expanded hexanucleotide repeat, resulting in the production and aggregation of dipeptide repeat (DPR) proteins (poly-GA, -GR and GP), was identified as a potential pathomechanism of C9ORF72 mutations. Besides accumulation of DPR proteins, the second neuropathological hallmark lesion in C9ORF72 mutation cases is the accumulation of TDP-43 [11-12].

From a genetic standpoint, familial antecedents are commonly observed in FTD. Interestingly, genetic analysis of familiar cases of FTD has revealed mutations in multiple loci including *GRN* [13-14] and *MAPT* [15] genes, both very close on chromosome 17, but also in *CHMP2B* [16], *UBQLN2* [17], *SQSTM1* [18]or *TREM2* [19] genes. Chromosome 9 open reading frame 72 (*C9ORF72*) mutation, located at chromosome 9p21 is characterized by a pathological non-coding hexanucleotide (G4C2) repeat expansion. This latter mutation within *C9ORF72* locus is the most frequent cause of inherited ALS and FTLD identified to date [20-22]. The isolation of these genes was a great success but, as yet, not all mutations underlying these proteinopathies have been identified [23]TAR DNA-binding protein TDP-43, fused-in-sarcoma or yet unidentified proteins in affected brain regions. Rather than the type of proteinopathy, the site of neurodegeneration correlates relatively well with the clinical presentation of FTLD. Molecular genetic studies identified five disease genes, of which the gene encoding the tau protein (MAPT. An international team led by University of Pennsylvania School of Medicine (USA), conducted a genome-wide association study (GWAS) in 515 subjects with autopsy-confirmed FTD with TDP-43 inclusions and 2509 neurologically normal controls to seek novel FTD-associated genes. All cases met either pathological or genetic criteria for FTD-TDP which was confirmed by detecting TDP-43 inclusions using immunohistochemistry (IHC) or mutation screening. Importantly, the authors found FTLD-TDP associates with multiple SNPs mapping in 7p21. This region contains the *TMEM106B* gene. Three SNPs retained genome-wide significance after multiple testing correction; top SNP rs1990622 minor allele (C) was reported as a protective genetic factor for FTD-TDP (OR=0.61, 95% CI 0.53-0.71). The association was replicated in 89 FTD-TDP autopsy-confirmed cases and 553 controls but not in a clinical FTD series available (a 192 FTD living patients cohort) [24]resulting from mutations in GRN (which encodes progranulin. Independent efforts to replicate this finding using clinical series have yielded controversial results. Rollinson et al (2011) attempted to replicate the association of these loci in FTD cohorts of British origin (520 FTD cases and 247 controls), but failed to detect any significant association of *TMEM106B* SNPs in the Manchester or London cohorts [3]. However, using an independent Flanders–Belgian cohort comprised primarily of clinically diagnosed patients (297 FTD cases and 595 controls), the association with *TMEM106B* rs1990622 [OR=0.75 (0.61–0.93)] was confirmed [25]. Finch et al, found a non-significant effect for *TMEM106B* in 640 FTD (clinical series) and 822 controls on FTD risk. It is noteworthy that a strong association between *TMEM106B* markers was detected in a small series of patients carrying *GRN* mutations but not in other FTD patients [26].

To evaluate the effect of *TMEM106B* rs1999622 on FTD risk, a new case-control association study was conducted in clinical FTD patients and neurologically normal controls. To conduct this research, the clinical FTD series available at Fundació ACE (Barcelona, Spain) were genotyped. A new meta-analysis integrating obtained results with currently available data was also performed.

MATERIALS AND METHODS

Patients

All of the patients agreed to participate in the study and blood samples were obtained after they and/or their legal representatives provided written consent according to the GIPSY protocol approved by the ethics committee in the Hospital Clinic i Provincial (Barcelona, Spain). Informed consent was in accordance with Spanish biomedical laws

(Law 14/2007, July 3rd, on biomedical research; Royal Decree 1716/2011, November 18th). All procedures involving experiments on human subjects are done in accordance with the ethical standards of the Ethics Committee of the institution in which the experiments were done or in accord with the Helsinki Declaration of 1975.

All patients were south-Europeans (Spanish lineage, two generation or more) recruited from the Fundació ACE. Barcelona Alzheimer Treatment & Research Center. Genotypes from 381 unrelated individuals were used for rs1990622 analysis (146 FTD cases, 234 neurologically normal controls). No individual has been included in any genome-wide dataset previously reported. 146 clinical FTD patients underwent a full neurological evaluation and were diagnosed using international research criteria: 88 bvFTD [1]an international consortium developed revised guidelines for the diagnosis of behavioural variant frontotemporal dementia. The validation process retrospectively reviewed clinical records and compared the sensitivity of proposed and earlier criteria in a multi-site sample of patients with pathologically verified frontotemporal lobar degeneration. According to the revised criteria, 'possible' behavioural variant frontotemporal dementia requires three of six clinically discriminating features (disinhibition, apathy/inertia, loss of sympathy/empathy, perseverative/compulsive behaviours, hyperorality and dysexecutive neuropsychological profile, 22 PNFA, 32 SD, [2]. Patients with Cortico-Basal Syndrome (CBS) or Progressive Supranuclear Palsy Syndrome (PSPS) [27]neuropsychology, and movement disorders specialists developed new criteria based on consensus and a systematic literature review. Clinical diagnoses (early or late, which in most cases are FTLD-TAU were excluded from this study, as were patients with Logopenic Aphasia (LPA) who were not genotyped, since cases LPA are mostly associated with a neuropathological diagnosis of AD [28-29]. Four Patients with a combined phenotype of FTD and ALS (FTD-MND) were also included. Patients were diagnosed at a daily consensus conference jointly by neurologists, neuropsychologists and social workers at the Fundació ACE Memory Clinic. Baseline signs and symptoms were acquired directly from the patient or from the primary caregiver. Patients were administered a neuropsychological battery that included measures of orientation , attention, verbal learning and long-term memory, language, gnosis, praxis and executive functions [30]. Neuroimaging studies used included CT and/or MRI, which were required for a probable FTD diagnosis according to revised criteria [1-2] ; in some cases SPECT scans were also available. The locus and extent of hemispheric atrophy was rated by visual inspection by an experienced neurologist, and independently confirmed by a neuroradiologist. FTD patients were screened looking for *MAPT* mutations and *C9ORF72* expansions. Only one FTD patient carrying a *MAPT* mutation (0.6%) was identified. Three additional patients were *C9ORF72* expansion carriers (1.8%) [31]frontotemporal lobar degeneration (FTLD. The remaining candidates genes were not studied. The exception was the *UBQLN2* gene that was partially screened in 77 FTD cases with familial antecedents of FTD. No mutation was detected in *UBQLN2* locus [32]. Mutation carriers were not excluded from this study.

Molecular analysis

DNA was extracted from frozen blood samples using the NucleoSpin Blood kit (Macherey-Nagel, Düren, Germany). The *TMEM106B* gene polymorphism rs1990622 was genotyped in an ECO Real-time PCR System (Illumina, San Diego, USA) by using the KAPA HRM fast PCR kit (Kapa Biosystems; Wobum, MA, USA) according to the manufacturer's instructions. High Resolution Melting primers were designed using DesignStudio online tool (Illumina; http://designstudio.illumina.com/). Selected amplification primers for High resolution melting were Forward: 5' CACTGTTTCATCCCCTTTATCTCGCCTA 3' and reverse: 5' TGCAGCTCTGTTCTCCATTGTAGTACTG 3'. The primer pair yields a 90 bp PCR product containing a unique polymorphism (rs1990622) according to Genome browser information. Real-time PCRs were performed in a reaction volume of 5 µl with 2.5 µl of HRM fast start master kit, 0,25µl of amplification primer mix directly purchased from Illumina (reference: 15028319), 0,5 µl of $MgCl_2$ (2.5mM end concentration; Kapa Biosystems), 10 ng of genomic DNA (2ng/ µl end concentration) and 0.75µl of PCR-grade water. Cycling conditions were as follows: 50°C for 2 minutes, 95°C for 10 minutes, and 45 cycles at 95°C for 10 seconds and 65°C during 15 seconds. Fluorescence was monitored at the end of each annealing/ extension phase. After amplification, specific conditions to obtain high resolution melting (HRM) curves were 95°C for 15 seconds, 55°C for 15 seconds, and 95°C for 15 seconds (ramping rate: 0.2°C/s). In the last step, continuous fluorimetric registers were performed by the system at one acquisition register per second. Melting peaks and genotype calls were obtained by using the Illumina Eco Real Time PCR system software version 4.1.02.0 (Illumina, San Diego, USA).

Statistical analysis and phenotype–genotype correlations.

Allelic frequencies, HWE equilibrium and genetic association studies were conducted using the online tool at TUM Helmholtz Center (Munich, Germany; http://ihg.gsf.de/cgi-bin/hw/hwa1.pl). We used tests adapted from the study conducted by Sasieni [33]there will be twice as many alleles as people. Another approach considers the risk of the disease in those who do not have the allele of interest (A. Age and sex-adjusted binary logistic regression analyses were performed using SPSS 15.0 software (SPSS, Chicago, IL). Meta-analyses were double checked using two independent online tools. We calculated estimates with Ken Rothman's Episheet spreadsheet (available online at http://www.krothman.org/episheet.xls).

Table 1
Association studies for rs1990622 in Fundació ACE case-control series using different genetic models.

Genetic model	Allele	Cell Counts (controls)	Cell counts (cases)	Effect (OR)	CI. 95%	p-value
Hardy-Weinberg Equilibrium	C/T	TT:95; TC:97; CC:42	TT:62;TC:68;CC:17	na	na	>0.053*
Allelic model (T vs C)	T	T:287; C:181	T:192; C:102	1.187	0.876-1.609	0.268
Heterozygous model (TC vs CC)	T	CC:42; TC:97	CC:17; TC:68	1.732	0.910-3.295	0.092
Homozygous model (TT vs CC)	T	CC:42; TT:95	CC:17; TT:62	1.612	0.843-3.082	0.146
Allele Positivy (TT+TC vs CC)	C	TT+TC:192; CC:42	TT+TC:130; CC:17	0.597	0.326-1.095	0.093
Armitage's Trend test	T	TT:95; TC:97; CC:42	TT:62;TC:68;CC:17	1.218	na	0.285

C: cytosine; T: thymine; OR: odds ratio; na: not applicable. *For Hardy-Weinberg calculations we conducted two tests (cases and controls separately). Allele positivity model is equivalent to T allele dominant model for or C allele recessive model.

The pooled estimate and 95% confidence intervals (CIs) were estimated by assuming fixed or random effects model (when necessary). The results were confirmed by using OpenMeta [Analyst] software freely available at Brown University (http://www.cebm.brown.edu/open_meta/download). Estimates were obtained from five different studies and eight independent datasets (2338 FTLD cases and 5248 controls). A total of 7586 samples were included in meta-analyses (468 cases and 553 controls from United Kingdom series; 640 cases and 822 controls from Mayo clinic study; 796 cases and 3062 controls from the original GWAS; 288 cases and 595 controls from Flanders-Belgian series and 146 cases and 234 controls from Fundació ACE). Forest plot with allelic and recessive (genotype CC) ORs from published studies were generated using OpenMeta [Analyst] software.

RESULTS

Rs1990622 Minor allele frequency (C allele; MAF=0.39 in controls), genotype distribution, and observed heterozygosity (43% for pooled sample) in this sample of the Spanish population are in accordance with the National Center for Biotechnology Information database and previous investigations. Hardy–Weinberg equilibrium analyses in our population indicated no gross deviations for this SNP (p=0.054 in controls; p=0.86 in FTD cases; p=0.15 pooled). The association of *TMEM106B* rs1990622 marker with FTD risk was explored subsequently using different tests (Table 1). No evidence of significant association was found with FTD in any comparison (P>0.09). Adjusted estimates using binary logistic regression models adjusting for age and sex were also obtained. Again, statistically significant effects on FTD risk were not detected in our series (P=0.138; data not shown). However, risk for FTD was higher for individuals carrying either one or two T alleles than CC carriers (OR=1.96 CI 95% [1-3.84], p=0.049 for one T-allele copy and OR=1.55 CI 95% [0.79-3.05], p=0.203 for two T-allele copies).

Overall, the *TMEM106B* rs1990622 CC genotype conferred a relative protection against FTD in this sample of the Spanish population. Of note, only a trend towards association was observed when age and sex-adjusted analysis of recessive model (genotype CC) was performed (adjusted OR=0.57; p=0.082). It is noteworthy that the observed effect size and direction were in line with previous results [24-25-26]resulting from mutations in GRN (which encodes progranulin. We also repeated the analysis by removing those individuals carrying a *MAPT* mutation (n=1) or *C9ORF72* expansions (n=3). The protective effect for genotype CC remained almost identical (OR=0.61; p=0.11). Interestingly, no patient carrying known mutations had the suggested protective genotype (CC) in rs1990622.

To further evaluate these observations, we decided to conduct a meta-analysis by using effect estimates data available from all previous publications [3-26-24] and our own results. By merging data from eight case–control studies and by using meta-analysis techniques, we were able to estimate the overall allelic effect of rs1990622, the effect in the replication series alone (excluding original studies for sensitivity analysis) and the genotype CC (in those studies with available genotypes or CC effect estimates) (Figure 1). Global meta-analysis resulted statistically significant (OR=0.80; C.I 95% [0.68-0.94]; p=0.0067; random effects model). However, we observed considerable heterogeneity among the studies (p=0.00014; Q-test; Figure 1a). This is not surprising taking into account that histopathological and clinical series were mixed in this meta-analysis. Importantly, after removing original results by Van Deerling et al. [24] resulting from mutations in GRN (which encodes progranulin and leaving only data from replication series, the heterogeneity disappeared (p=0.54; Q-test) and the effect remained quite similar and statistically significant (OR=0.88; C.I.95% [0.80-0.96]; p=0.0092) (Figure 1b). Most importantly, pooled analysis also suggested that recessive CC genotype model might work better than allelic model for rs1990622 by gaining one order of magnitude in p-value (OR=0.70; C.I. 95% [0.57-0.85]; p=0.0003; Figure 1c) with no evidence of heterogeneity among available studies (p=0.36).

Phenotype-genotype correlation was also conducted in the Fundació ACE series by dividing clinical FTD cases according to rs1990622 genotypes (genotypic, recessive or dominant models) (tables 2 and 3). We did not find any evidence of association between characteristics clinical

Figure 1A: Overall Meta-analysis (Random effects)

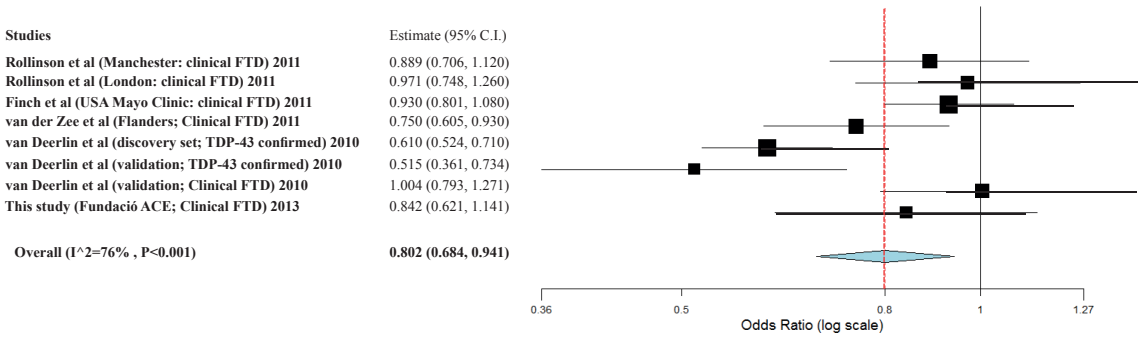


Figure 1B: Sensitivity Analysis (excluding original results)

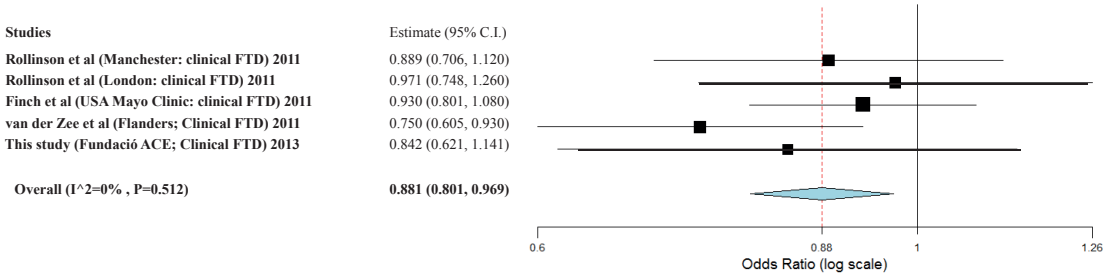


Figure 1C: Meta-analysis (CC-recessive model)

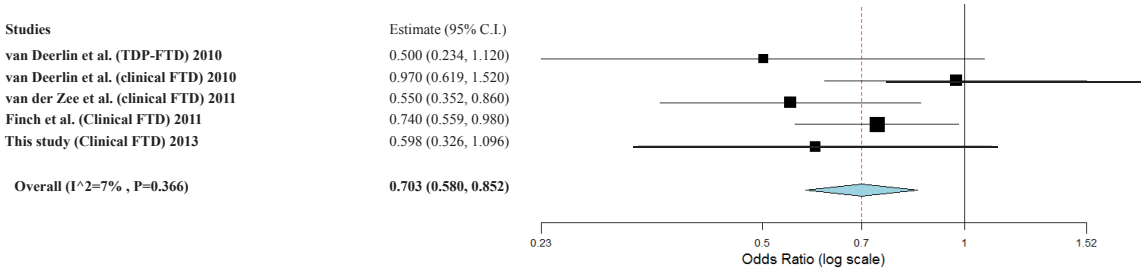


Fig. l. Fixed and random effects model meta-analyses and Forest plots of rs1990622, reporting odds ratio (OR) with 95% confidence interval (Cl). A) All available studies, random effects model for allele C. B) Meta-analyses and Forest plots using replication studies only (sensitivity analysis; fixed effects model for allele C). C) Meta-analyses and Forest plots for genotype CC (recessive genetic effect).

patients and rs1990622 genotypes after the application of multiple testing corrections. However, it is noteworthy that evidence of familial antecedents was less frequent in patients carrying CC genotype than non-CC individuals (p=0.03). Moreover, overall phenotype-genotype analyses suggested that rs1990622 SNP cannot be used to differentiate any clinical subgroup of FTD patients.

The results revealed a non-significant protective effect for rs1990622 C allele in our population. Meta-analysis using previously available datasets and the results obtained pointed to the existence of genetic association of rs1990622 SNP to FTD risk.

DISCUSSION

We observed a trend toward significant results on analyzing the *TMEM106B* effect on FTD risk in our FTD clinical series. Furthermore, we estimated that in terms of pathology characteristics only a percentage, presumably 50%, of our FTD cases would be eligible for inclusion in a hypothetical study of only TDP-43 positive FTD cases.

Table 2
Demography of Patients with clinical FTD randomized by rs1990622 SNP (genotypic, dominant and recessive models).

	All patients *n*=146	rs1990622bi			Dominant Model C		Recessive model C	
		CC	TC	TT	TT	TC/CC	No CC	CC
Age at onset , x (SD)	68,5 (8,9)	66.3 (11.2)	68 (8.0)	69.1(9.4)	69.1 (9.4)	67.7 (8.7)	68.5 (8.7)	66.3 (11.2)
MMSE, x (SD)	22,5 (6,1)	21.4 (7.7)	22.3 (5.0)	22.7 (6.6)	22.7 (6.6)	22.1 (5.7)	22.5 (5.9)	21.4 (7.7)
Male N (%)	79 (54.1)	12 (15)	31 (38.8)	37 (46.3)	37 (46.3)	43 (53.8)	68 (85.0)	12 (15.0)
Familiar History	72 (49.3)	4 (23.5)	35 (54.7	29 (46.8)	29 (46.8)	39 (48.1)	64 (50.8)	4 (23.5)
Clinical phenotype								
bvFTD**	88 (60.3)	10 (11.4)	36 (40.9)	42 (47.7)	42 (47.7)	46 (52.3)	78 (88.6)	10 (11.4)
PNFA	22 (15.1)	0	13 (59.1)	9 (40.9)	9 (40.9)	13 (59.1)	22 (100)	0
SD	32 (21.9)	5 (15.6)	16 (50)	11(34.7)	11 (34.4)	21 (65.6)	27 (84.4)	5(15.6)
FTD-MND	4 (2.7)	2 (50.0)	2 (50.0)	0	0	4 (100)	2 (50)	2 (50)

bvFTD: behavioral variant Frontotemporal dementia; PNFA: Progressive non fluent aphasia ; SD: Semantic Dementia; FTD-MND: Frontotemporal dementia with motorneuron disease. **Familiar History (*p*: 0.038) for bvFTD in recessive model C

In any event, a trend toward association was observed for C-allele protection on risk. This observation is very similar to the effect observed in a Flanders-Belgian clinical FTD cohort [25]. Crude and adjusted analyses pointed to the idea that effect on risk of rs1990622 C-allele might work as a recessive protection allele in our population.

Lack of statistical significance cannot be over-interpreted under these circumstances. In fact, bearing in mind our limitations in terms of sample size and phenotypic heterogeneity, we do consider that a genuine effect of the *TMEM106B* markers on FTD risk exists. In fact, we obtained a similar estimation of effect in our population compared to previous clinical series (figure 1).

Importantly, meta-analysis and sensitivity analysis confirmed our results. This strategy may help to corroborate genetic signals with a suspected smaller or even a diluted effect. This was the case with rs1990622 SNP. In fact, by merging data from fairly negative studies such as van Deerlin's clinical FTD series or the British case-control studies, we obtained statistically significant results through meta-analysis (Figure 1a,b,c). The exclusion of original results did not affect the meta-analysis. Therefore, sensitivity analysis also supports the existence of a genuine effect on FTD risk for this locus.

The results obtained with regard to the case, or patient selection during the genome-wide studies must be discussed. First, from a diagnostic standpoint, SNPs derived from GWAS conducted in pathologically-confirmed series might be very difficult to validate using clinically based studies. Therefore, it will be necessary to have clinical datasets with large sample sizes to verify pathology-based GWAS results. It is noteworthy that this phenomenon could be the standard for dozens of weak GWAS signals obtained in multiple studies of complex diseases. Subsequently, beyond the well-recognized winner's curse, usually affecting any replication effort [34]odds ratio (OR, the dilution of effect in clinical series must be taken into account for the design of GWAS replication programs aiming to confirm isolated GWAS signals by using pathology datasets . The concordant effect of rs1990622 observed in this study supported the notion that datasets with a reduced sample sizes might be benefited from the increasing of clinical homogeneity and from the reduction of ethnic stratification. This could be the case of Fundació ACE dataset compared to other clinical series that failed to detect the protective effect of rs1990622.

In our opinion the dilution effect of *TMEM106B* SNP in clinical series seems evident (Figure 1a and b). The observed differences in effect estimation between studies and heterogeneity could be attributable to selection of FTD cases. Importantly, clinical FTD might also contain Tau and FUS- related FTD cases (about 50%). Pathology heterogeneity could explain the dilution effect observed in all clinical series studied to date (figure 1). Consequently, we reason that the strong heterogeneity observed might have appeared by merging pathological and clinical series. This phenomenon reinforces the notion that pathology-driven GWAS could be an excellent strategy to purify genuine genetic factors affecting only very specific patient subgroups. However, isolated genes will be masked in any attempt at replication using clinical series. This dilution effect could be one of the reasons explaining the percentage of heritability still uncovered after extensive application of meta-GWAS strategies [35]. Having this in mind, a pathology-driven case selection could be an advisable strategy to isolate hidden loci for other neurodegenerative disorders with contrasted pathology heterogeneity.

The lack of information of our series in *GRN* locus represents a serious limitation of this study. It has been suggested that the *TMEM106B* effect could be restricted only to patients carrying *GRN* mutations [26]. However, original findings showed a significant association with FT-LD-TDP, both in those with and without *GRN* mutations [24]resulting from mutations in GRN (which encodes

progranulin. Moreover, the frequency of *GRN* mutations in Spanish FTD patients is quite low (<6%) [36]8 asymptomatic mutation carriers, and 10 control subjects as well as in brain tissue from 16 patients and 9 control subjects. RESULTS: Four novel mutations were associated with familial and sporadic FTLD and familial dementia associated with amyotrophic lateral sclerosis. We identified a close association between the IVS6-1G>A mutation in PGRN and corticobasal syndrome. Brain tissue was available for carriers of two of the four mutations (IVS6-1 G>A and P357HfsX3. This data makes it improbable that the obtained results in our series and meta-analysis could be only attributed to *GRN* mutation carriers. In regard to FTD diagnosis, it will be necessary to determine which genetic backgrounds are affected or modulated by *TMEM106B* markers. However, data derived from other studies pointed to the idea that *GRN* mutation penetrance could be modulated by this locus [26-37]. Interestingly, the only significant phenotypic characteristic of FTD patients that might be observed in patients carrying rs19906622-CC was a reduced frequency of familial antecedents. This finding is consistent with a higher effect of this locus in *GRN* mutation carriers previously observed. However, the *TMEM106B* gene might modify other FTD mutations. As a recent publication suggested, *TMEM106B* genotype could act as a genetic modifier of the penetrance of *C9ORF72* expansions. The observation was restricted only to those patients displaying FTD clinical phenotype alone or in combination with MND. Notably, the effect on *C9ORF72* expansion was more evident assuming a recessive model which was consistent with our meta-analysis [38]586 FTD patients lacking C9ORF72 expansions [with or without motor neuron disease (MND. This observation, when replicated, would confirm that *TMEM106B* locus effect is not restricted to FTD patients carrying GRN mutations. Further research and independent replications are needed to corroborate these clinical observations.

In conclusion, the observed heterogeneity and statistical significance in the results observed during meta-analysis point to the idea that the *TMEM106B* locus is a genuine modifying genetic factor at least in a subgroup of FTD-TDP cases. Unfortunately, only a pathologically-confirmed FLTD series would help to clarify this point. However, the problem could be re-visited once radiotracers for distinct subtypes of FTD pathology become available. Finally, the molecular mechanism explaining observed association is still under investigation [26-34-35]. Recent functional studies suggested its role in progranulin pathways by affecting *GRN* degradation in lysosomal pathways [39]. This information might be relevant in the design of novel therapeutic strategies for FTD-TDP.

ACKNOWLEDGEMENTS

We thank donors and their families for generous blood donation for research. This work was carried out as part of the doctoral program of I. Hernández at the Autonomous University of Barcelona. This work was partially supported by the Spanish Ministry of Health from Instituto de Salud Carlos III (Madrid) (FISS PI10/00945) and by the Agència d'Avaluació de Tecnologia i Recerca Mèdiques. Departament de Salut de la Generalitat de Catalunya (Health Department of the Catalan Government) (390). We are indebted to Trinitat Port-Carbó and her family for their support of the Fundació ACE research programs.

REFERENCES

[1] Rascovsky K, Hodges J. R, Knopman D, Mendez MF, Kramer JH, Neuhaus J, van Swieten JC, Seelaar H, Dopper EGP, Onyike CU, Hillis AE, Josephs KA, Boeve BF, Kertesz A, Seeley WW, Rankin KP, Johnson JK, Gorno-Tempini ML, Rosen H, Prioleau-Latham CE, Lee A, Kipps C M, Lillo P, Piguet O, Rohrer JD, Rossor MN, Warren JD, Fox NC, Galasko D, Salmon DP, Black SE, Mesulam M, Weintraub S, Dickerson BC, Diehl-Schmid J, Pasquier F, Deramecourt V, Lebert F, Pijnenburg Y, Chow TW, Manes F, Grafman J, Cappa SF, Freedman M, Grossman M, Miller BL. (2011) "Sensitivity of revised diagnostic criteria for the behavioural variant of frontotemporal dementia. *Brain* **134** (Pt 9), 2456–2477.

[2] Gorno-Tempini ML, Hillis AE, Weintraub S, Kertesz A, Mendez M, Cappa SF, Ogar JM, Rohrer JD, Black S, Boeve BF, Manes F, Dronkers NF, Vandenberghe R, Rascovsky K, Patterson K, Miller BL, Knopman DS,. Hodges JR, Mesulam MM, Grossman M. (2011) "Classification of primary progressive aphasia and its variants. *Neurology* **76** (11), 1006–1014.

[3] Rollinson S, Mead S, Snowden J, Richardson A, Rohrer J, Halliwell N, Usher S, Neary D, Mann D, Hardy J, Pickering-Brown S. (2011) Frontotemporal lobar degeneration genome wide association study replication confirms a risk locus shared with amyotrophic lateral sclerosis. *Neurobiology of aging* **32** (4), 758.e1–7.

[4] Achi EY, Rudnicki SA. (2012) ALS and Frontotemporal Dysfunction: A Review. *Neurology research international* 2012, Article ID 806306, 9 pages.

[5] Boxer A L, Mackenzie IR, Boeve BF, Baker M, Seeley WW, Crook R, Feldman H, Hsiung GYR, Rutherford N, Laluz V, Whitwell J, Foti D, McDade E, Molano J, Karydas A, Wojtas A, Goldman J, Mirsky J, Sengdy P, Dearmond S, Miller BL, Rademakers R. (2011) Clinical, neuroimaging and neuropathological features of a new chromosome 9p-linked FTD-ALS family. *Journal of neurology, neurosurgery, and psychiatry* **82** (2), 196–203.

[6] Piguet O, Halliday GM, Reid WGJ, Casey B, Carman R, Huang Y, Xuereb JH, Hodges JR, Kril JJ. (2011)Clinical phenotypes in autopsy-confirmed

Table 3

Clinical characteristics of Patients with clinical FTD randomized by rs1990622 SNP (genotypic, dominant and recessive models)

All patients		Rs1990622bi				Dominant Model C			Recessive Model C		
		CC	TC	TT	*p*	TT	TC/CC	*p*	No CC	CC	*p*
	Neuroimaging n(%).										
27 (22.7)	Frontal atrophy	3 (15.0)	9 (45.0)	8 (40.0)	0.750	8 (40.0)	12(60.0)	0.461	17(85.0)	3 (15.0)	0.691
35 (29.4)	Temporal atrophy	2 (16.7)	4 (33.3)	6 (50.0)		6 (50.0)	6 (50.0)		10 (83.3)	2 (16.7)	
3 (2.5)	Parietal atrophy	0	1(100)	0		0	1 (100)		1 (100)	0	
41 (34.5)	Frontotemporal atrophy	3 (9.4)	11 (34.4)	18 (56.3)		18 (56.3)	14 (43.8)		29 (90.6)	3 (9.4)	
10 (8.4)	Global atrophy	2 (33.3)	3 (50.0)	1 (16.7)		1 (16.7)	5 (83.3)		4 (66.7)	2 (33.3)	
3 (2.5)	Frontoparietal atrophy	0	1 (50.0)	1 (50.0)		1 (50.0)	1 (50.0)		2 (100)	0	
	Predominant alteration signs at baseline reported by caregiver n (%)										
73 (50.7)	Language	9 (12.3)	36 (55.4)	28 (38.4)	0.505	28 (38.4)	45 (6.6)	0.313	64 (87.7)	9 (12.3)	1
105 (42.9)	Memory/Attention	13 (12.4)	49 (46.7)	43(41.0)	0.702	43 (41.0)	62 (59.0)	0.451	92 (87.6)	13 (12.4)	1
96 (66.7)	Behavioral	14 (14.6)	38 (39.6)	44 (45.8)	0.113	44 (45.8)	52 (52.2)	0.376	82 (85.4)	14 (14.6)	0.178
	Pathological background n(%)										
45 (33.3)	Hypertension	5 (11.1)	22 (48.9)	18 (40.0)	0.928	18 (40.0)	27 (60.0)	0.854	40 (88.9)	5 (11.1)	1
49 (36.0)	Dyslipidemia	6 (12.2)	21 (42.9)	22 (44.9)	0.828	22(44.9)	27 (55.1)	0.718	43 (87.8)	6 (12.2)	1
16 (11.8)	Diabetes mellitus	1 (6.3)	9(56.3)	6 (37.5)	0.625	6 (37.5)	10 (62.5)	0.792	15 (93.8)	1 (6.3)	0.693
26 (18.1)	Heart disease	3 (11.5)	8 (30.8)	15 (57.7)	0.151	15 (57.7)	11 (42.3)	0.077	23 (88.5)	3 (11.5)	1
8 (5.9)	Minor Stroke	1 (12.5)	2 (25.2)	5 (62.5)	0.414	5(62.5)	3 (37.8)	0.276	7 (87.5)	1 (12.5)	1
	Neuropsychological variables altered N (%)										
76 (61.3)	Total orientation	8 (10.7)	37(48.7)	31 (40.8)	0.468	31 (40.8)	45 (59.2)	0.267	68 (89.5)	8 (10.5)	0.765
17 (14.0)	Language fluency	2 (11.8)	11 (64.7)	4 (23.5)	0.139	4 (23.5)	13 (76.5)	0.066	15 (88.2)	2 (11.8)	0.676
93 (75.0)	Boston naming (15-BNT)	9 (9.7)	42 (45.2)	42 (45.2)	1	42 (45.2)	51 (54.8)	1	84 (90.3)	9 (9.7)	1
95 (66.0)	Praxis altered	7 (7.4)	48 (50.5)	40 (42.1)	0.147	40 (42.1)	55 (57.9)	0.448	88 (92.6)	7 (7.4)	0.158
97 (80.2)	Learning memory WMS-III	9 (9.3)	46 (47.4)	42 (43.3)	0.459	42 (43.3)	55 (56.7)	0.368	88 (90.7)	9 (9.3)	0.703
91 (76.5)	Recognition memory WMS-III	9 (9.9)	44 (48.4)	38 (41.8)	0.166	38 (41.3)	53 (58.2)	0.088	82 (90.1)	9 (9.9)	1
88 (75.9)	PVF	8 (9.1)	41 (46.6)	39 (44.3)	0.528	39 (44.3)	49 (55.7)	0.599	80 (90.9)	8 (9.1)	0.480
93 (78.2)	SVF	10 (10.8)	43 (46.2)	40 (43.0)	0.609	40 (43.0)	53 (57.0)	0.377	83 (89.2)	10(10.8)	1
99 (87.6)	Abstract reasoning	10(10.1)	45 (45.5)	44 (44.4)	0.487	44 (44.4)	55 (55.6)	0.404	89 (89.9)	10(10.1)	0.643
103 (85.1)	Direct digits	10 (9.7)	47 (45.6)	46 (44.7)	0.868	46 (44.7)	57 (55.3)	0.799	93 (90.3)	10 (9.7)	1
90 (74.4)	Reverse digits	7 (7.8)	41 (45.6)	42 (46.7)	0.406	42 (46.7)	48 (53.3)	0.681	83 (92.2)	7 (7.8)	0.183
67 (62.6)	SKT time	6 (9.0)	23 (58.8)	35 (52.2)	0.399	35 (52.2)	32 (47.8)	0.237	61 (91.0)	6 (9.0)	0.358
52 (55.9)	SKT error	5 (9.6)	21 (40.4)	26 (50.0)	0.555	26 (50.0)	26 (50.0)	0.676	47 (90.4)	5 (9.6)	0.287
54 (47.4)	Clock test	7 (13.0)	25 (46.3)	22 (40.7)	0.535	22 (40.7)	32 (59.3)	0.351	47 (46.1)	7 (58.3)	0.545

Global orientation: summa of Temporal+Spatial+Personal orientations; WMS-III: Wechsler Memory Scale, Third Edition; The abbreviated BNT: Boston Naming Test with 15 visual items; Recognition memory: correct answers; Block Design: WAIS-III;SKT: Automatic Inhibition Symdrom Kurztest; PVF: Phonemic verbal fluency; SVF: Semantic verbal fluency

Pick disease. *Neurology* **76** (3), 253–259.

[7] Piguet O, Hornberger M, Mioshi E, Hodges JR. (2011) Behavioural-variant frontotemporal dementia: diagnosis, clinical staging, and management. *Lancet neurology* **10** (2), 162–172.

[8] Roeber S, Mackenzie IRA, Kretzschmar HA, Neumann M. (2008) TDP-43-negative FTLD-U is a significant new clinico-pathological subtype of FTLD. *Acta neuropathologica* **116** (2), 147–157.

[9] Mackenzie IRA, Munoz DG, Kusaka H, Yokota O, Ishihara K, Roebe S, a Kretzschmar H, Cairns NJ, Neumann M. (2011) Distinct pathological subtypes of FTLD-FUS. *Acta neuropathologica* **121** (2), 207–218.

[10] Mackenzie IRA, Neumann M, Bigio EH, Cairns NJ, Alafuzoff I, Kril J, Kovacs GG, Ghetti B, Halliday G, Holm IE, Ince PG, Kamphorst W, Revesz T, Rozemuller AJM, Kumar-Singh S, Akiyama H, Baborie A, Spina S, Dickson DW, Trojanowski JQ, A. Mann DM. (2009) Nomenclature for neuropathologic subtypes of frontotemporal lobar degeneration: consensus recommendations. *Acta neuropathologica* **117** (1), 15–18.

[11] Mori K, Weng SM, Arzberger T, May S, Rentzsch K, Kremmer E, Schmid B, Kretzschmar HA, Cruts M, Van Broeckhoven C, Haass C, Edbauer D. (2013) The C9orf72 GGGGCC repeat is translated into aggregating dipeptide-repeat proteins in FTLD/ALS. *Science* **339** (6125), 1335–1338.

[12] Mackenzie IR, Arzberger T, Kremmer E, Troost D, Lorenzl S, Mori K, Weng SM, Haass C, Kretzschmar HA, Edbauer D, Neumann M. (2013) Dipeptide repeat protein pathology in C9ORF72 mutation cases: clinico-pathological correlations. *Acta neuropathologica* **126** (6), 859–879.

[13] Baker M, Mackenzie IR, Pickering-Brown SM, Gass J, Rademakers R, Lindholm C, Snowden J, Adamson J, Sadovnick AD, Rollinson S, Cannon A, Dwosh E, Neary D, Melquist S, Richardson A, Dickson D, Berger Z, Eriksen J, Robinson T, Zehr C, Dickey CA, Crook R, McGowan E, Mann D, Boeve B, Feldman H, Hutton M. (2006) Mutations in progranulin cause tau-negative frontotemporal dementia linked to chromosome 17 *Nature* **442** (7105), 916–919.

[14] Cruts M, Kumar-Singh S, Van Broeckhoven C. (2006) Progranulin mutations in ubiquitin-positive frontotemporal dementia linked to chromosome 17q21. *Current Alzheimer research* **3** (5), 485–491.

[15] Hutton M, Lendon CL, Rizzu P, Baker M, Froelich S, Houlden H, Pickering-Brown S, Chakraverty S, Isaacs A, Grover A, Hackett J, Adamson J, Lincoln S, Dickson D, Davies P, Petersen RC, Stevens M, de Graaff E, Wauters E, van Baren J, Hillebrand M, Joosse M, Kwon JM, Nowotny P, Che LK, Norton J, Morris JC, Reed LA, Trojanowski J, Basun H, Lannfelt L, Neystat M, Fahn S, Dark F, Tannenberg T, Dodd PR, Hayward N, Kwok JB, Schofield PR, Andreadis A, Snowden J, Craufurd D, Neary D, Owen F, Oostra BA, Hardy J, Goate A, van Swieten J, Mann D, Lynch T, Heutink P. (1998) Association of missense and 5'-splice-site mutations in tau with the inherited dementia FTDP-17. *Nature* **393** (6686), 702–705.

[16] Skibinski G, Parkinson NJ, Brown JM, Chakrabarti L, Lloyd SL, Hummerich H, Nielsen JE, Hodges JR, Spillantini MG, Thusgaard T, Brandner S, Brun A, Rossor MN, Gade A, Johannsen P, Sørensen SA, Gydesen S, Fisher EM, Collinge J (2005). Mutations in the endosomal ESCRTIII-complex subunit CHMP2B in frontotemporal dementia. *Nature genetics* **37** (8), 806–808.

[17] Deng HX, Chen W, Hong ST, Boycott KM, Gorrie GH, Siddique N, Yang Y, Fecto F, Shi Y, Zhai H, Jiang H, Hirano M, Rampersaud E, Jansen GH, Donkervoort S, Bigio EH, Brooks BR, Ajroud K, Sufit RL, Haines JL, Mugnaini E, Pericak-Vance MA, Siddique T. (2011) Mutations in UBQLN2 cause dominant X-linked juvenile and adult-onset ALS and ALS/dementia. *Nature* **477** (7363), 211–215.

[18] Rubino E, Rainero I, Chiò A, Rogaeva E, Galimberti D, Fenoglio P, Grinberg Y, Isaia G, Calvo A, Gentile S, Bruni AC, St George-Hyslop PH, Scarpini E, Gallone S, Pinessi L; TODEM Study Group .(2012) SQSTM1 mutations in frontotemporal lobar degeneration and amyotrophic lateral sclerosis *Neurology* **79** (15),1556–1562.

[19] Guerreiro RJ, Lohmann E, Brás JM, Gibbs JR, Rohrer JD, Gurunlian N, Dursun B, Bilgic B, Hanagasi H, Gurvit H, Emre M, Singleton A, Hardy J .(2013) Using exome sequencing to reveal mutations in TREM2 presenting as a frontotemporal dementia-like syndrome without bone involvement. *JAMA neurology* **70** (1), 78–84.

[20] DeJesus-Hernandez M, Mackenzie IR, Boeve BF, Boxer AL, Baker M, Rutherford NJ, Nicholson AM, Finch NA, Flynn H, Adamson J, Kouri N, Wojtas A, Sengdy P, Hsiung GY, Karydas A, Seeley WW, Josephs KA, Coppola G, Geschwind DH, Wszolek ZK, Feldman H, Knopman DS, Petersen RC, Miller BL, Dickson DW, Boylan KB, Graff-Radford NR, Rademakers R. (2011) Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. *Neuron* **72** (2), 245–256.

[21] Renton AE, Majounie E, Waite A, Simón-Sánchez J, Rollinson S, Gibbs JR, Schymick JC, Laaksovirta H, van Swieten JC, Myllykangas L, Kalimo H, Paetau A, Abramzon Y, Remes AM, Kaganovich A, Scholz SW, Duckworth J, Ding J, Harmer DW, Hernandez DG, Johnson JO, Mok K, Ryten M, Trabzuni D, Guerreiro RJ, Orrell RW, Neal J, Murray A, Pearson J, Jansen IE, Sondervan D, Seelaar

H, Blake D, Young K, Halliwell N, Callister JB, Toulson G, Richardson A, Gerhard A, Snowden J, Mann D, Neary D, Nalls MA, Peuralinna T, Jansson L, Isoviita VM, Kaivorinne AL, Hölttä-Vuori M, Ikonen E, Sulkava R, Benatar M, Wuu J, Chiò A, Restagno G, Borghero G, Sabatelli M; ITALSGEN Consortium, Heckerman D, Rogaeva E, Zinman L, Rothstein JD, Sendtner M, Drepper C, Eichler EE, Alkan C, Abdullaev Z, Pack SD, Dutra A, Pak E, Hardy J, Singleton A, Williams NM, Heutink P, Pickering-Brown S, Morris HR, Tienari PJ, Traynor BJ (2011). A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. *Neuron* **72** (2), 257–268.

[22] Gijselinck I, Van Langenhove T, van der Zee J, Sleegers K, Philtjens S, Kleinberger G, Janssens J, Bettens K, Van Cauwenberghe C, Pereson S, Engelborghs S, Sieben A, De Jonghe P, Vandenberghe R, Santens P, De Bleecker J, Maes G, Bäumer V, Dillen L, Joris G, Cuijt I, Corsmit E, Elinck E, Van Dongen J, Vermeulen S, Van den Broeck M, Vaerenberg C, Mattheijssens M, Peeters K, Robberecht W, Cras P, Martin JJ, De Deyn PP, Cruts M, Van Broeckhoven C. (2012) A C9orf72 promoter repeat expansion in a Flanders-Belgian cohort with disorders of the frontotemporal lobar degeneration-amyotrophic lateral sclerosis spectrum: a gene identification study. *Lancet neurology* **11** (1), 54–65.

[23] Sieben A, Van Langenhove T, Engelborghs S, Martin JJ, Boon P, Cras P, De Deyn PP, Santens P, Van Broeckhoven C, Cruts M. (2012) The genetics and neuropathology of frontotemporal lobar degeneration. *Acta neuropathologica* **124** (3), 353–372.

[24] Van Deerlin VM, Sleiman PM, Martinez-Lage M, Chen-Plotkin A, Wang LS, Graff-Radford NR, Dickson DW, Rademakers R, Boeve BF, Grossman M, Arnold SE, Mann DM, Pickering-Brown SM, Seelaar H, Heutink P, van Swieten JC, Murrell JR, Ghetti B, Spina S, Grafman J, Hodges J, Spillantini MG, Gilman S, Lieberman AP, Kaye JA, Woltjer RL, Bigio EH, Mesulam M, Al-Sarraj S, Troakes C, Rosenberg RN, White CL 3rd, Ferrer I, Lladó A, Neumann M, Kretzschmar HA, Hulette CM, Welsh-Bohmer KA, Miller BL, Alzualde A, Lopez de Munain A, McKee AC, Gearing M, Levey AI, Lah JJ, Hardy J, Rohrer JD, Lashley T, Mackenzie IR, Feldman HH, Hamilton RL, Dekosky ST, van der Zee J, Kumar-Singh S, Van Broeckhoven C, Mayeux R, Vonsattel JP, Troncoso JC, Kril JJ, Kwok JB, Halliday GM, Bird TD, Ince PG, Shaw PJ, Cairns NJ, Morris JC, McLean CA, DeCarli C, Ellis WG, Freeman SH, Frosch MP, Growdon JH, Perl DP, Sano M, Bennett DA, Schneider JA, Beach TG, Reiman EM, Woodruff BK, Cummings J, Vinters HV, Miller CA, Chui HC, Alafuzoff I, Hartikainen P, Seilhean D, Galasko D, Masliah E, Cotman CW, Tuñón MT, Martínez MC, Munoz DG, Carroll SL, Marson D, Riederer PF, Bogdanovic N, Schellenberg GD, Hakonarson H, Trojanowski JQ, Lee VM. (2010) Common variants at 7p21 are associated with frontotemporal lobar degeneration with TDP-43 inclusions. *Nature genetics* **42** (3), 234–239.

[25] van der Zee J, Van Langenhove T, Kleinberger G, Sleegers K, Engelborghs S, Vandenberghe R, Santens P, Van den Broeck M, Joris G, Brys J, Mattheijssens M, Peeters K, Cras P, De Deyn PP, Cruts M, Van Broeckhoven C. (2011) TMEM106B is associated with frontotemporal lobar degeneration in a clinically diagnosed patient cohort. *Brain* **134** (Pt 3), 808–815.

[26] Finch N, Carrasquillo MM, Baker M, Rutherford NJ, Coppola G, Dejesus-Hernandez M, Crook R, Hunter T, Ghidoni R, Benussi L, Crook J, Finger E, Hantanpaa KJ, Karydas AM, Sengdy P, Gonzalez J, Seeley WW, Johnson N, Beach TG, Mesulam M, Forloni G, Kertesz A, Knopman DS, Uitti R, White CL 3rd, Caselli R, Lippa C, Bigio EH, Wszolek ZK, Binetti G, Mackenzie IR, Miller BL, Boeve BF, Younkin SG, Dickson DW, Petersen RC, Graff-Radford NR, Geschwind DH, Rademakers R. (2011) TMEM106B regulates progranulin levels and the penetrance of FTLD in GRN mutation carriers. *Neurology* **76** (5), 467–474.

[27] Armstrong MJ, Litvan I, Lang AE, Bak TH, Bhatia KP, Borroni B, Boxer AL, Dickson DW, Grossman M, Hallett M, Josephs KA, Kertesz A, Lee SE, Miller BL, Reich SG, Riley DE, Tolosa E, Tröster AI, Vidailhet M, Weiner WJ. (2013) Criteria for the diagnosis of corticobasal degeneration. *Neurology* **80** (5), 496–503.

[28] Rabinovici GD, Jagust WJ, Furst AJ, Ogar JM, Racine CA, Mormino EC, O'Neil JP, Lal RA, Dronkers NF, Miller BL, Gorno-Tempini ML. (2008) Abeta amyloid and glucose metabolism in three variants of primary progressive aphasia. *Annals of neurology* **64** (4), 388–401.

[29] Pan XD, Chen XC. (2013) Clinic neuropathology and molecular genetics of frontotemporal dementia: a mini-review. *Translational neurodegeneration* **2** (1):8.

[30] Alegret M, Espinosa A, Vinyes-Junqué G, Valero S, Hernández I, Tárraga L, Becker JT, Boada M. (2012) Normative data of a brief neuropsychological battery for Spanish individuals older than 49. *Journal of clinical and experimental neuropsychology* **34** (2), 209–219.

[31] Xi Z, Zinman L, Grinberg Y, Moreno D, Sato C, Bilbao JM, Ghani M, Hernández I, Ruiz A, Boada M, Morón FJ, Lang AE, Marras C, Bruni A, Colao R, Maletta RG, Puccio G, Rainero I, Pinessi L, Galimberti D, Morrison KE, Moorby C, Stockton JD, Masellis M, Black SE, Hazrati LN, Liang Y, van Haersma de With J, Fornazzari L, Villagra R, Rojas-Garcia R, Clarimón J, Mayeux R, Robertson J,

St George-Hyslop P, Rogaeva E. (2012) Investigation of C9orf72 in 4 Neurodegenerative Disorders. *Archives of neurology* **69** (12),1583-1590

[32] Hernández I, Espinosa A, Real LM, Galán JJ, Mauleón A, Roca MR, Tárraga L, Ruiz A, Boada M. (2012) Molecular evaluation of human Ubiquilin 2 gene PXX domain in familial frontotemporal dementia patients. *Journal of neurology* **259** (11), 2488–2490.

[33] Sasieni PD. (1997) From genotypes to genes: doubling the sample size. *Biometrics* **53** (4), 1253–1261.

[34] Zhong H, Prentice RL. (2010) Correcting 'winner's curse' in odds ratios from genomewide association findings for major complex human diseases. *Genetic epidemiology* **34** (1), 78–91.

[35] Manolio TA, Collins FS, Cox NJ, Goldstein DB, Hindorff LA, Hunter DJ, McCarthy MI, Ramos EM, Cardon LR, Chakravarti A, Cho JH, Guttmacher AE, Kong A, Kruglyak L, Mardis E, Rotimi CN, Slatkin M, Valle D, Whittemore AS, Boehnke M, Clark AG, Eichler EE, Gibson G, Haines JL, Mackay TF, McCarroll SA, Visscher PM. (2009) Finding the missing heritability of complex diseases. *Nature* **461** (7265),747–753.

[36] A. López de Munain, A. Alzualde, A. Gorostidi, D. Otaegui, J. Ruiz-Martínez, B. Indakoetxea, I. Ferrer, J. Pérez-Tur, A. Sáenz, A. Bergareche, M. Barandiarán, J. J. Poza, R. Zabalza, I. Ruiz, M. Urtasun, I. Fernández-Manchola, B. Olasagasti, J. B. Espinal, J. Olaskoaga, M. Ruibal, F. Moreno, N. Carrera, and J. F. Martí Massó. (2008) Mutations in progranulin gene: clinical, pathological, and ribonucleic acid expression findings. *Biological psychiatry* **63** (10), 946–952.

[37] Cruchaga C, Graff C, Chiang HH, Wang J, Hinrichs AL, Spiegel N, Bertelsen S, Mayo K, Norton JB, Morris JC, Goate A. (2011) Association of TMEM106B gene polymorphism with age at onset in granulin mutation carriers and plasma granulin protein levels. *Archives of neurology* **68** (5), 581–586.

[38] van Blitterswijk M, Mullen B, Nicholson AM, Bieniek KF, Heckman MG, Baker MC, DeJesus-Hernandez M, Finch NA, Brown PH, Murray ME, Hsiung GY, Stewart H, Karydas AM, Finger E, Kertesz A, Bigio EH, Weintraub S, Mesulam M, Hatanpaa KJ, White CL 3rd, Strong MJ, Beach TG, Wszolek ZK, Lippa C, Caselli R, Petrucelli L, Josephs KA, Parisi JE, Knopman DS, Petersen RC, Mackenzie IR, Seeley WW, Grinberg LT, Miller BL, Boylan KB, Graff-Radford NR, Boeve BF, Dickson DW, Rademakers R. (2014) TMEM106B protects C9ORF72 expansion carriers against frontotemporal dementia. *Acta neuropathologica* **127 (**3), 397–406.

[39] Brady OA, Zheng Y, Murphy K, Huang M, Hu F. (2013) The frontotemporal lobar degeneration risk factor, TMEM106B, regulates lysosomal morphology and function. *Human molecular genetics* **22** (4), 685–695.

VIII. Discusión

En este trabajo de tesis se constata la necesidad de integrar la colaboración entre la labor que desempeña el investigador clínico y los resultados que aporta el laboratorio de análisis genético, puesto que en este estadio de la investigación la presencia del genotipo causal que explica la patología ya comienza a ser un dato habitual disponible, que requiere una interpretación apropiada.

La resultados entre lo que el clínico valora en su contacto diario con los pacientes, en base a su experiencia, protocolos y criterios clínicos, son datos cualitativos no exentos de error, mientras que el profesional de la genética interpreta en su muestreo ADN resultados cuantitativos y estadísticos, si bien la sobre-interpretación de los hallazgos genéticos también deben tenerse en cuenta. Por ello, es esencial una base de datos clínica rigurosa y de calidad para la identificación de los nuevos loci asociados a cada patología, a la que sumar la mejora de la interpretación de los hallazgos clínicos gracias a la incorporación de la información genética. Una estrecha colaboración entre ambos profesionales es indispensable para una mejor comprensión de las bases que conforman las enfermedades neurodegenerativas.

Como se ha descrito anteriormente, la DFT es un grupo de enfermedades neurodegenerativas que se presentan de forma diferente, tanto desde el punto de vista clínico, como genético y neuropatológico. (Figura 2 y Figura 3).

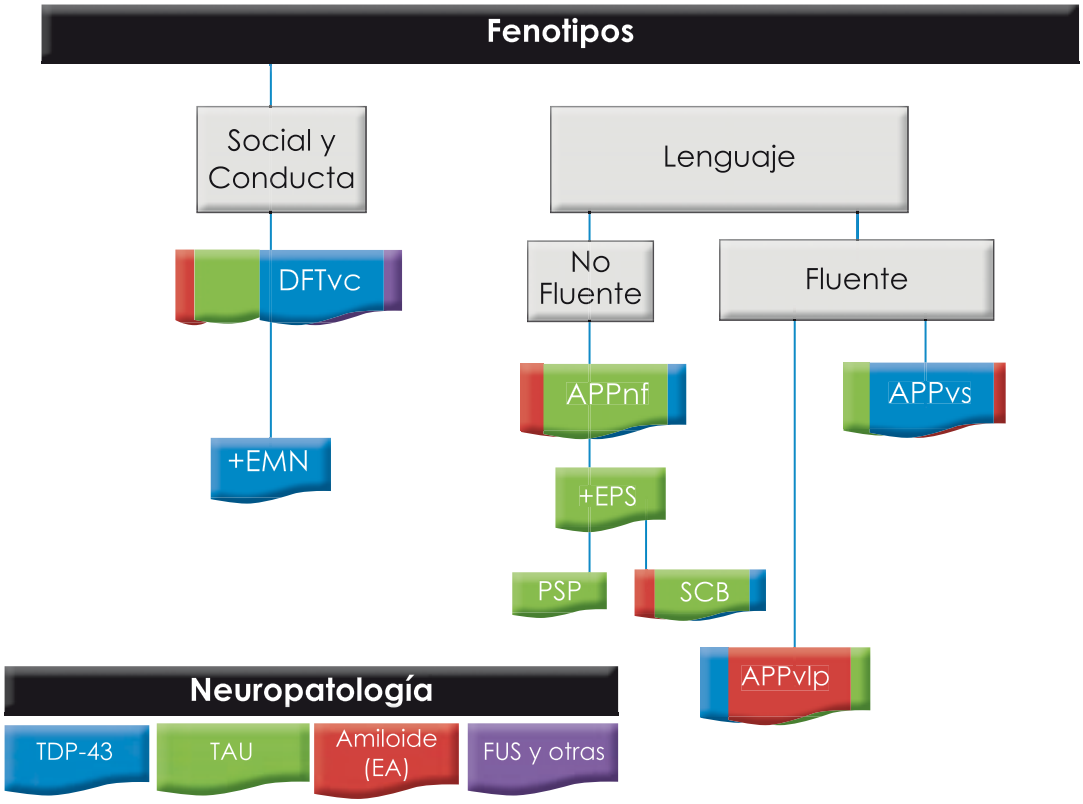


Figura 2: Fenotipos Clínicos y correlación patológica

DFTvc: Demencia Frontotemporal variante de conducta. **EMN**: Enfermedad de motoneurona. **APPnf**: Afasia Progresiva no fluente. **EPS**: Síndrome Extrapiramidal. **PSP**: Parálisis Supranuclear Progresiva. **SCB**: Síndrome Cortico Basal. **APPvs**: Afasia Progresiva variante semántica. **APPvlp**: Afasia Progresiva Primaria variante logopénica. **TDP-43**: transactive response DNA binding protein 43 kDa. **TAU**: Proteína Tau, **EA**: Enfermedad de Alzheimer. **FUS**: Fused in Sarcoma/Translocated in Sarcoma

(Adaptado de Hernández et al. Manuscrito en preparación)

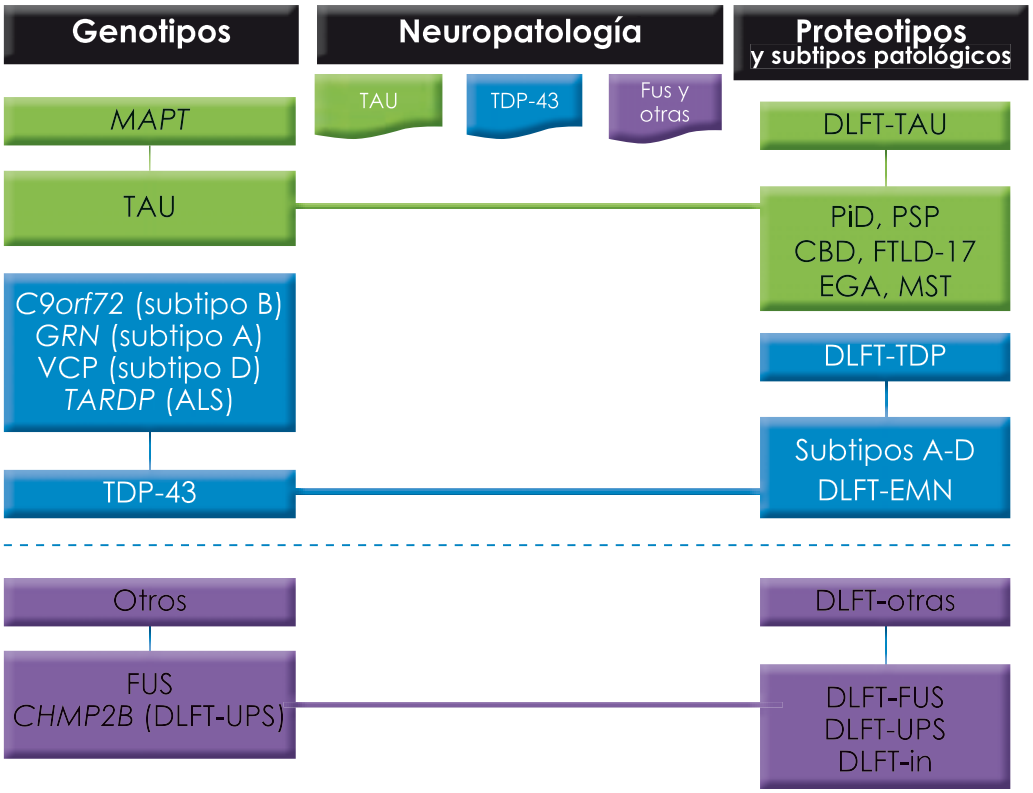


Figura 3: Genotipos DLFT y correlación patológica

.*MAPT*: Microtubule associated protein Tau gen. **TAU**: Proteína Tau. **DLFT-TAU**: Demencia Lobar Frontotemporal por depósitos de proteína Tau, **PiD**: Enfermedad de Pick. **PSP**: Parálisis Supranuclear Progresiva. **DCB**: Degeneración Cortico Basal. **FTLD-17**: Demencia Frontotemporal con parkinsonismo asociado al Cr17. **EGA**: Enfermedad por Granos Argirófilos. **MST**: Taupatía Multisistémica. **DLFT-TDP**: Demencia Lobar Frontotemporal con inclusiones de proteína TDP-43. ***C9orf72***: chromosome 9 open reading frame 72. ***GRN***: Gen Progranulina. ***VCP***: Gen Valosina. ***TARDP***: gen TAR DNA Binding Protein. **TDP-43**: transactive response DNA binding protein 43 kDa. **DLFT-EMN**; Demencia Frontotemporal con Enfermedad de motoneurona asociada. **FUS**: gen Fused in Sarcoma. ***CHMP2B***: gen Charged multivesicular body protein. **DLFT-UPS**: Degeneración Lobar Frontotemporal con inmuno-histoquímica contra proteínas del sistema ubiquitina-proteasoma, **DLFT-FUS**: Degeneración Lobar Frontotemporal por depósitos de proteína FUS. **DLFT-in**: Degeneración Lobar Frontotemporal sin inclusiones.

(Adaptado de Hernández et al. manuscrito en preparación)

Respecto a la caracterización de la serie Clínica

La serie clínica utilizada en este trabajo de tesis ha sido un trabajo de observación y seguimiento clínico realizado a lo largo de 15 años. Los pacientes han sido valorados clínicamente con instrumentos comunes y seguidos en la mayoría de casos por el autor hasta su defunción. Esto ha permitido observar y objetivar la progresión y el cambio en algunos de sus fenotipos y disponer de información evolutiva de cada sujeto. Del mismo modo se ha obtenido un porcentaje aceptable de confirmaciones patológicas de una misma serie. El seguimiento continúa en muchos de los sujetos.

La evolución fenotípica de los 224 sujetos, desde su diagnóstico inicial, se muestra en Tabla 3. Clínicamente, los fenotipos evolutivos muestran que la DFTvc es el más frecuente, con el 50% de casos (n=112) (Rascovsky et al. 2011), seguido de la DS con el 15.2% (n=34) y la APNF con el 9.4% (n=21), lo que suponen el 24% de casos con fenotipo APP (M L Gorno-Tempini et al. 2011). El resto de formas de presentación clínica las encontramos en el SCB con el 9.8% (n=22) (Armstrong et al. 2013) y la SPSP con el 10.7% (n=24)(Litvan et al. 1996). Sólo en el 3.6% (n=8) encontramos fenotipo DFT-EMN y en todos los casos en forma de DFTvc, probablemente porque, si su debut clínico es en forma de ELA son valorados en primera instancia en las Unidades específicas de Sistema Nervioso Periférico. Estos resultados son concordantes con lo que se observa en otras series (Johnson et al. 2005b). De los ocho pacientes inicialmente orientados como EA, 5 (62.5%) de ellos evolucionaron a DFTvc y 3 (37.5%) hacia SCB. Así mismo, una APNF y 2 DFTvc en diagnóstico inicial, evolucionaron clínicamente a EA.

En 7 casos de APNF (23%) la evolución permitió observar la presentación de un SPSP en 4 casos y de SCB en 3 casos, confirmándose en dos casos APNF de inicio, con evolución a SCB, una Taupatía de 4R. (I Ferrer et al. 2003) (Anexo).

Atendiendo a los datos de neuroimagen, observamos un predominio del patrón frontotemporal con 53 sujetos (29.4%) seguido del patrón frontal en 47 sujetos (26.1%) y el temporal en 44 (24.4%), lo que suma un 80% de la serie y donde podríamos incluir los fenotipos DFTvc y las APP (Tabla 4). El restante 20% presenta un patrón de atrofia difuso o frontoparietal. En este % probablemente están representados los fenotipos SPSP, con un patrón más global y el SCB con un patrón más característico frontoparietal.

De toda la serie clínica a estudio se han conseguido hasta el momento 31(13.8%) confirmaciones anatomopatológicas (Tabla 5), donde el proteotipo Tau y TDP-43 suponen el 74.3 % de los casos. El proteotipo amiloide está presente en el 19.4% de la serie.

Tabla 3: Fenotipo evolutivo de la serie DFT clínica

Diagnóstico Basal	Fenotipo Evolutivo N (%)							
	N (%)	DFTvc	APNF	DS	DFT-EMN	SCB	PSP	EA
Psiquiátrico	3 (1.3)	2 (66.7)	--	1 (33.3)	--	--	--	--
SCB	14 (6.3)	--	1 (7.1%)	--	--	12 (85.7)	1 (7.1)	--
PSP	16 (7.1)	--	--	--	--	--	16 (100)	--
EA	8 (3.6)	5 (62.5)	--	--	--	3 (37.5)	--	--
DCL	17 (7.6)	11 (64.7)	1 (5.9)	2 (11.8)	1 (5.9)	2 (11.8)	--	
APNF	32 (14.3)	3 (9.4)	19 (59.4)	1 (3.1)	1 (3,6)	3 (9.4)	4 (12.5)	1 (3.1)
DFTvc	99 (44.2)	88 (88.9)	--	1 (1.0)	4 (4.0)	1 (1.0)	3 (3.0)	2 (2.0)
DS	33 (14.7)	3 (9.1)	--	29 (87.9)	0	1 (3.0)	--	--
DFT-EMN	2 (0.9)	--	--	--	2 (100)	--	--	--
Total N (%)	224	112 (50.0)	21 (9.4)	34 (15.2)	8 (3.6)	22 (9.8)	24 (10.7)	3 (1.3)

Tabla 4: Patrones de Neuroimagen

Diagnóstico Basal	Atrofia en Neuroimagen N (%)						
	N (%)	Frontal	Temporal	Parietal	Fron-to-tem-poral	Global	Fron-to-parie-tal
Psiquiátrico	3 (1.7)	1 (33.3)	1 (33.3)	--	1 (3.3	--	--
SCB	9 (5.0)	1 (11.1)	1 (11.1)	1 (11.1)	2 (22.2)	2 (22.2)	2 (22.2)
PSP	12 (6.7)	3 (25.0)	--	1 (8.3)	2 (16.7)	6 (50.0)	--
EA	6 (3.3	1 (16.7)	--	1 (16.7)	3 (50.0)	1 (16.7)	--
DCL	11 (6.1)	3 (27.3)	2 (18.2)	--	5 (45.5)	--	1 (9.1)
APNF	27 (15.0)	9 (3.3)	6 (22.2)	2 (7.4)	5 (18.5)	4 (14.8)	1 (3.7)
DFTvc	82 (45.6)	27 (32.9)	15 (18.3)	2 (2.4)	28 (34.1)	9 (11.0)	1 (1.2)
DS	30 (16.7)	2 (6.7)	19 (63.3)	--	7 (23.3)	2 (6.7)	--
Total	180	47 (26.1)	44 (24.4)	7 (3.9)	53 (29.4)	24 (13.3)	5 (2.8)

Tabla 5: Neuropatología disponible de la serie clínica

Fenotipo al éxitus N (%)		Neuropatología N (%)						
		Amiloide	Tau 4R	Tau 3R	α-sincucleina	Cuerpos argirófilos	TDP-43	IF-α-internexina
DFTvc	10 (32.3)	2 (20.0)	1 (10.0)	1 (10.0)	--	1 (10.0)	4 (40.0)	1(10.0)
SCB	6 (19.4))	2 (33.3)	2 (33.3)	--	1 (16.7)	--	1 (16.7)	--
PSP	5 (16.1)	--	5 (100)	--	--	--	--	--
DFT-ELA	6 (19.4)	1 (16.7)	--	--	--	1 (16.7)	4 (66.7)	--
APNF	2 (6.5)	--	2 (100)	--	--	--	--	--
DS	2 (6.5)	1 (50)	--	--	--	--	1(50)	--
Total	31	6 (19.4)	10 (32.3)	1 (3.2)	1 (3.4)	2 (6.5)	10 (32.3)	1 (3.2)

La caracterización y el diagnóstico clínico de cada síndrome van a depender de la realización de una anamnesis detallada, investigando en profundidad, generalmente con la persona informante, los signos iniciales del proceso. La identificación de los síntomas del paciente en las etapas iniciales será crucial de cara a establecer posibles tratamientos médicos y proporcionar el mejor seguimiento y soporte familiar a cada caso.

Respecto al análisis genético de la serie

a. Objetivos relacionados con la tesis

Trabajo I: *UBQLN2*

Se examinaron 77 pacientes con historia familiar de demencia que poseían evaluación clínica completa. Es importante destacar que mantuvimos los pacientes con DFT de transmisión varón-varón (donde la trasmisión dominante ligada al X es imposible) como controles negativos de este estudio (n = 12).

No se encontraron mutaciones de *UBQLN2* en nuestra población, por lo que la correlación genotipo-fenotipo fue desestimada.

Trabajo II: *C9orf72*

Los datos fueron obtenidos gracias a la colaboración con el *"Tanz Center for Research in Neurodegenerative Diseases. Universidad de Toronto. Canadá"* que realizó el estudio multicéntrico y la determinación de las mutaciones *C9ofr72*. (Xi et al. 2012).

Para este trabajo se aportaron a la serie a estudio 159 muestras de ADN. De ellas 102 (64.6%) sujetos con fenotipo DFTvc, 3 (1.9%) fenotipo DFT-EMN, 22 (13.9%) con fenotipo APPnf y 31 (19.6%) con fenotipo DS. A estos hay que añadir la confirmación de la expansión G_5C_2 positiva posmortem de otro caso con fenotipo DFTvc:

► En los sujetos con fenotipo APPnf y DS (N: 53) no se obtuvieron expansiones positivas.

► De los 106 sujetos restantes, 5.7%, eran portadores de C9*orf72*: 83% fenotipo DFTvc y 16.6% fenotipo DFT-EMN, lo que concuerda con trabajos publicados (Hsiung et al. 2012), donde la expansión de C9orf72 sólo se ha observado en los fenotipos DFTvc y ELA.

► Las dos AP de las que se disponen, con fenotipo DFTvc ambas, fueron concluyentes con depósitos de TDP-43, proteotipo característico de la expansión.

Casos de la serie clínica de Fundació ACE publicados en el artículo. Descripciones (extraídas directamente de la información suplementaria del artículo (Xi et al. 2012).

► "M0010497. *A 71 year old man with a 5 year history of memory problems manifested as losing objects and getting lost in the neighborhood. About 4 years prior, the patient's wife assumed responsibility of his business affairs. Currently, the patient continues to suffer with memory problems with some spatial/time disorientation. The patient is completely dependent on his wife and is homebound, is not aggressive and does not suffer from delusions or hallucinations. However, the patient shows impulsive behavior with a tendency to compulsively buy food and shows a lack of initiative. Neuropsychological assessment concluded that the patient was disoriented to reality and he showed an impairment of attention, language, visual gnosis, praxis and prefrontal functions.*

►*M0024815. A 50 year old man with a strong history of dementia (father with bvFTD, grandfather and two paternal uncles with dementia) began to develop irritability and difficulty with executive function. Shortly after diagnosis, he stopped working. There were no problems with language. He is still able to carry out basic day to day functions without supervision. The neuropsychological assessment showed a significant slowing of cognitive processing and a severe impairment of verbal learning, verbal working memory, complex visual gnosis, visuoconstructive praxis, visuospatial abilities, inhibition of automatic responses and action verbal fluency. There was a mild impairment of attention, verbal free recall and recognition memory, writing, complex verbal comprehension, phonemic verbal fluency and abstract reasoning. In contrast, he showed a relative preservation of spontaneous speech, confrontational object naming, repetition of sentences, reading, simple visual gnosis, imitational and ideomotor praxis, and category verbal fluency.*

►*M0018127. A 61 year old woman with a family history of dementia (sister and father) showed behavioral changes and memory loss at age 60. She sings excessively and speaks with the characters on the television. She is obsessed with money and is impulsive. She frequently calls in to TV contests and makes inappropriate comments. However, she is still able to function independently most of the time. The neuropsychological assessment showed impairment of visual gnosis and visuoconstructive praxis; and a mild impairment of attention, verbal working memory, inhibition of automatic responses and abstract reasoning.*

► *M0010098. A 66 year old man with family history of dementia (brother and father) began to suffer from memory problems and disorientation at age 60. The family notes that at that time he was unable to carry out normal activities of daily living. At present, the patient presents with temporal and spatial disorientation, language difficulties, apathy, sleep disorder, bulimia, and a sexual disorder. The neuropsychological assessment showed significant slowing of cognitive processing and a severe impairment of attention, verbal learning, verbal working memory, spontaneous speech (tendency to mutism, stereotyped behaviour), writing, reading, complex verbal comprehension, confrontation object naming, visual gnosis, imitation, inhibition of automatic responses, phonemic and category verbal fluency, and abstract reasoning. There was also a mild impairment of verbal free recall and recognition memory (Figure 4).*

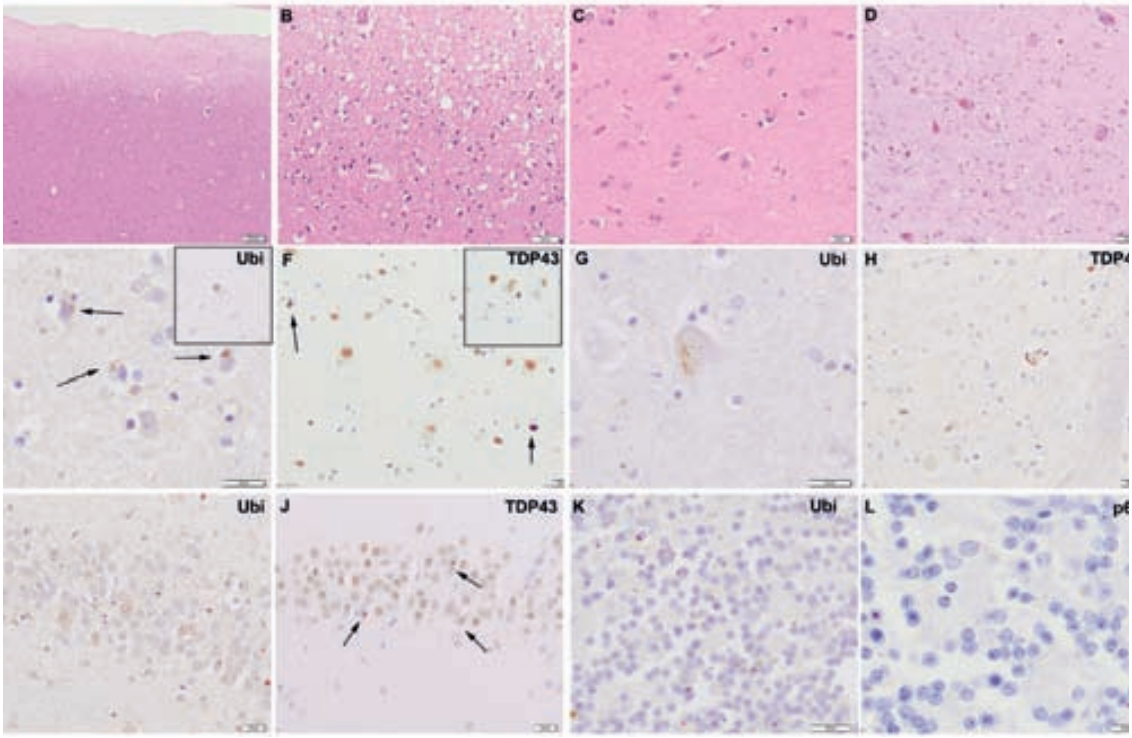


Figura 4: Neuropathology of pacient M0010098

A-D: Hematoxylin-eosin stains showing moderate neuronal loss and gliosis in frontal cortex (A) associated to prominent microvacuolation of upper cortical laminae (B). There is in addition marked neuronal loss and gliosis of caudate nucleus (C). In D, a moderate reduction of neuromelanin-laden neurons of substantia nigra is observed, with extracellular pigment, gliosis and macrophages. E-L: Immunohistochemistry shows the presence of frequent small neuronal cytoplasmic inclusion bodies mainly immunoreactive for ubiquitin in frontal cortex (E), dentate gyrus of hippocampus (I) and granular neurons of cerebellar cortex (K). These are also immunoreactive for p62 (L). Some round neuronal nuclear inclusions are detected in basal ganglia (inset in E) and cerebellar granule cells (L, left of the image). Some ramified, skein-like inclusions are detected in some neurons of the n. ruber in the midbrain (G). TDP43-immunoreactive neuronal cytoplasmic inclusions are detected in frontal cortex (F, arrows), some of them with skein-like morphology (inset in F), in granule cells of dentate gyrus of the hippocampus (J) and single neurons of the inferior olivary nucleus (H). No TDP-43 positive inclusions are observed in cerebellar granule cell layer (not shown).

Trabajo III: Polimorfismo *ApoE*

No se ha demostrado que la *ApoE4* sea un polimorfismo de riesgo para la DFT, aunque numerosos artículos han intentado mostrar asociación. Esto posiblemente sea debido a que todas las series clínicas de DFT están contaminadas con sujetos que neuropatológicamente eran EA aunque su evolución clínica mostrara criterios de DFT.

En este trabajo se han combinado las variables clínicas la serie de DFT con el genotipado de *ApoE* y los casos con confirmación patológica. La hipótesis ha sido establecida con la premisa de crear un algoritmo discriminante que nos permitiera caracterizar aquellos casos EA atípicos que están erróneamente clasificados como DFT. La metodología utilizada se describe ampliamente en el artículo.

Remarcar que el genotipado de *ApoE* sólo se utilizó en un primer paso, durante el análisis discriminante, para escoger las 14 variables clínicas que mostraban una tendencia estadística para discernir portadores y no portadores de ε4 dentro de la serie clínica. 30 casos de la serie fueron asignados por el discriminante a EAvf (EA variante frontal) y se compararon fenotípicamente con el resto de sujetos de la serie DFT, obteniendo los siguientes datos:

► El 26.3% de los 30 sujetos asignados a EAvf eran portadores de ε4 y de éstos, el 80 %, revisada su evolución fenotípica, mostraron signos de evolución a EA, pero de características atípicas (SCB, ACP, APPvl). El clasificador no discriminó en los casos SPSP.

► Se halló una frecuencia más alta de cambio de diagnóstico a lo largo del curso de la enfermedad en EAvf comparados con el resto (31% versus 16.9%; p=0.01), lo que indica un fenotipo evolutivo más inestable.

►En la visita basal, el 90% de los clasificados como EA manifestaban quejas de memoria frente al 34 % de las DFT.

► Los sujetos que el LDA selecciona como EA son de mayor edad y con más carga de *ApoE* (ε4: 21% <65 años vs 29.6% en >65 años).

► El fenotipo DFTvc es más frecuente en los más jóvenes (53% <65 años vs 36 % > 65 años).

► Como síntomas de inicio, la alteración de memoria estaba presente en el 26.7 % <65 frente al 46.3 >65 años (p= 0.018) y la conducta en el 61.4 % <65 frente al 38.0 >65 años (p= 0.007).

Trabajo IV: *TMEN106B*

Un GWAS realizado en 515 sujetos con confirmación anátomo-patológica de DLFT-TDP, llevado a cabo por el equipo de la Universidad de Pennsylvania en 2010,(Deerlin et al. 2010) mostraba el efecto protector del SNP rs1990622 del gen TMEM106B (localizado en 7p21). En concreto, el alelo menos frecuente (C), (los autores asignaron el alelo C como un factor protector y modificador de riesgo de la DLFT-TDP. Otros estudios han tratado de replicar dichos resultados, usando series clínicas, pero han mostrado resultados controvertidos (para detalles véase la introducción). La causa de esta controversia puede ser debida a que en las series clínicas DFT sólo el ≥50% pacientes son DLFT-TDP.

En este trabajo se ha estudiado el SNP rs19990622 en nuestra serie DFT clínica con tres modelos diferentes (genotípico, dominante y recesivo). Teniendo en consideración el número de la muestra (147 DFT y 234 controles), se optó por realizar un meta-análisis con los resultados, asociándolos a los datos disponibles de publicaciones previas y una correlación fenotipo-genotipo entre los diferentes subtipos de DFT.

► Aplicando el modelo recesivo (CC) en rs1990622 se observó una tendencia a la asociación con el riesgo de DFT (ajustado por edad y sexo) con un OR =0.57; p= 0.082

► El meta-análisis también apoyó el efecto del modelo recesivo para el genotipo rs1990622 (OR =0.70; CI 95% [0.57-0.85] p=0.0003).

► Se observó que el riesgo para DFT era mayor para los individuos portadores de uno o dos alelos T (definidos como de riesgo) que para los portadores de CC (OR=1.96 CI 95% [1-3.84], p=0.049 para una copia del alelo T y OR=1.55 CI 95% [0.79-3.05] para dos copias del alelo T.

► La correlación fenotipo-genotipo llevada a cabo, para los tres modelos de randomización, por genotipo, recesivo y dominante, no mostró evidencia de asociación en ninguna de las variables clínicas utilizadas, salvo en los antecedentes familiares de demencia, que fueron menos frecuentes en los individuos DFTvc portadores del genotipo de protección CC (p=0.03).

b. Otros genes de la serie clínica estudiados.

TAU* y *GRN

Estos genes, de carácter autosómico dominante, sólo se han estudiado en aquellos sujetos en los que se sospechaba una clara historia familiar mendeliana. Estos trabajos se realizaron en colaboración con el Institut de Recerca-Hospital de la Santa Creu i Sant Pau y dentro de otro proyecto de investigación paralelo.

Para el estudio se han aportado 14 muestras de la serie, encontrando únicamente una mutación de TAU, ya conocida previamente. No se detectaron mutaciones en Progranulina en ninguna de las muestras,

El paciente con la mutación TAU detectada (P310L) presentaba un fenotipo DLFT-17 (Foster et al. 1997) que debutó con una DFTvc a los 45 años y posteriormente desarrolló signos parkinsonianos típicos. En la Figura 5 se puede observar la AP, que por las características especiales que presentaba fue objeto de publicación (Isidro Ferrer et al. 2003) (Anexo)

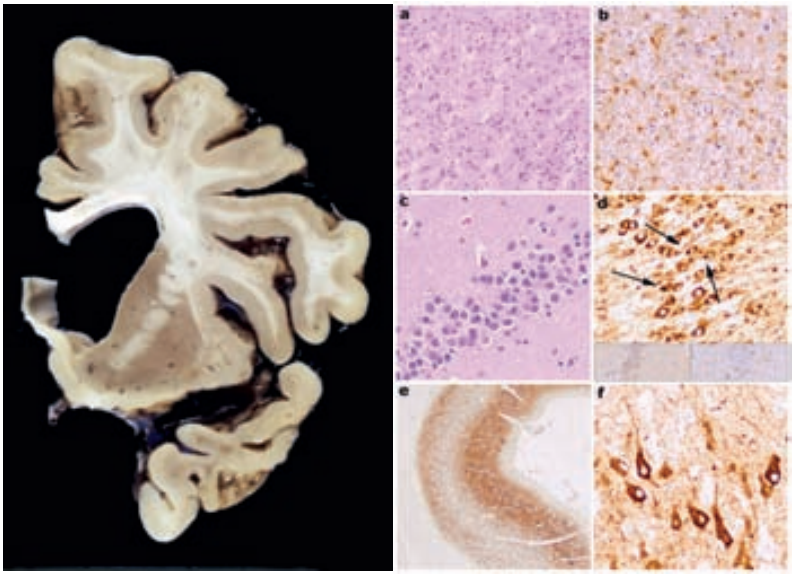


Figura 5: Neuropatología del paciente con mutación TAU

Cortesia de la Dra. Ellen Gelpí. IDIBAPS. Banco de Tejidos Neurologicos. Universidad de Barcelona

Características neuropatológicas representativas de la mutación P301L MAPT que muestran atrofia cerebral grave, patrón de degeneración lobar fronto-temporal con reducción de circunvoluciones, aplanamiento del núcleo caudado y dilatación ventricular marcada (imagen macroscópica izquierda). La histología muestra pérdida neuronal generalizada con espongiosis (a) y gliosis prominente (b, inmunohistoquímica con (GFAP) proteína glial fibrilar ácida) en las áreas afectadas del cerebro asociadas con la acumulación intraneuronal extensa de la proteína tau hiperfosforilada (e) en forma de inmunoreactividad citoplasmática difusa (pretangles), hebras de neuropilo y ovillos neurofibrilares (f) y pequeñas inclusiones globulares extensas en las neuronas granulares de la circunvolución dentada del hipocampo (mini-Pick-like bodies; flechas en (d), que son inmunorreactivas para isoformas Tau de 3R y 4R (pequeñas inserciones en d).

SQSTM1

Este es un proyecto multicéntrico realizado en colaboración con el Departamento de Genética Molecular VIB de (Amberes, Bélgica). Se aportaron 147 (8%) muestras a un total de 1808 casos de DFT y 234 (6%) muestras a un total de 3899 controles (van der Zee et al. 2014). No se encontraron mutaciones en nuestra serie.

En la muestra total se identificaron 25 variantes raras en heterocigosis que afectaban a la región codificante de *SQSTM1*, que incluían 23 mutaciones por cambio de aminoácido, una mutación nula que interrumpía la pausa de lectura y una pequeña deleción que no alteraba la pauta de lectura. No se encontraron estas mutaciones en los en 3.899 controles.

TREM2

Trabajo de colaboración con DEGESCO (Dementia Genetics Spanish Consortium) con la aportación de 539 muestras DFT (148 de esta serie) y 666 controles y el "*Department of Psychiatry and Psychotherapy, y Institute of Human Genetics. University of Bonn, Germany*" con la aportación de 63 muestras DFT y 939 controles.

De nuestra serie clínica se incluyeron 148 sujetos (24.5%) y 234 controles (14.5%). Se identificó un paciente con la mutación W44X, publicada en 2002 (Paloneva BM et al. 2001) como causante de displasia poliquística lipomenbranosa con leucoencefalopatia esclerosante (PLOST, Enfermedad de Nasu-Hakola) y 5 variantes que a través del análisis bioinformático se infieren como patogénicas (Tabla 6).

Tabla 6: Variantes TREM2 raras identificadas en la serie y fenotipo asociado

Casos	1	2	3	4	5	6	7	8	9	10
Años	75	72	71	74	86	77	77	64	68	78
Género	Varón	Mujer	Varón	Varón	Varón	Mujer	Varón	Mujer	Varón	Mujer
Mutación	W44X	R62H	A28V	A105T	R62H	T96K	R62H	T96K	R47H	R47H
Interpretación	Patogénica	Patogénica	No patogénica	No patogénica	No patogénica	Patogénica	No patogénica	Patogénica	Patogénica	Patogénica
Síntoma de inicio	Conducta	Conducta	Lenguaje	Lenguaje	Conducta	Lenguaje	Memoria	Disejecutivo	Disejecutivo	Lenguaje
Fenotipo evolutivo	DFTvc	DFTvc	APP-PSP	DS-DF-Tvc	DFTvc	DS	DFTvc	DFT-EMN	APPvl	APPvl

GWAS : Estudio de asociación de genoma completo de DFT

A este estudio, realizado en colaboración con "*The National Institute of Neurological Disorders and Stroke and National Institute on Aging, the Wellcome/MRC Centre on Parkinson's disease, Alzheimer's Research UK, and Texas Tech University Health Sciences Center*" se aportaron 237 controles y 155 muestras de la serie clínica: 99 DFTvc (63%), 22 APPnf (14%), 31 APPvs (20%) Y 3 DFT-ELA (2%) a un total de 2154 pacientes con DFT y 4308 controles. Nuestra serie se empleo para la fase de replicación y los genotipos están disponibles para estudios futuros.

Se han identificado 2 nuevos genes que superan el umbral de significación para estudio de genoma completo. Los loci identificados son el *HLA* en la posición 6p 21.3 y un marcador del gen*RAB38/CTSC* en la posición 11p14.

Los resultados sugieren que las alteraciones de los procesos del sistema inmune (6p21.3) y, posiblemente, de la fisiología lisosomal y vías de la autofagia, (11q14) podrían estar implicados en la DFT.

IX. Conclusiones

1. A nivel fenotípico se observa una variabilidad en la evolución clínica de la serie DFT. De ello se desprende la necesidad de un seguimiento clínico a largo plazo para identificar claramente los fenotipos, siendo, como en todas las series clínicas publicadas, el fenotipo DFTvc el más prevalente.

2. A nivel genético, se ha observado que la expansión del *C9orf72* es la mutación más frecuente dentro de la serie. Todos los portadores presentan el fenotipo DFTvc y/o ELA. Se confirma que la expansión de *C9orf72* es la mayor causa de DFT en nuestra serie, de causa familiar y la primera a estudiar en esta variante fenotípica.

3. Dentro de los polimorfismos estudiados se concluye que la ApoE E4 no está asociada a la DFT y que dentro de las series clínicas DFT hay que sopesar la probable contaminación de fenotipos EA. Los resultados están en concordancia con la hipótesis de que casos EA variante frontal están inflando la frecuencia alélica de ApoE4 dentro de las series DFT y esto podría ayudar a diferenciarlos clínicamente; corroborando esta observación, la existencia de "información clínica de EA" dentro de la serie DFT.

4. Respecto al polimorfismo *TMEM106B*, nuestros resultados indican que aplicando el modelo recesivo (CC) se observa una tendencia a la asociación en relación con el riesgo de DFT (ajustado por edad y sexo, con una odds ratio=0.57; p=0.082). Los meta-análisis de los estudios disponibles también apoyan el efecto recesivo para el genotipo CC rs1990622 (OR=0.70; C.I. 95% [0.57-0.85]; p=0.0003). Así, concluimos que *TMEM106B* está asociado con DFT, aunque el grado de este efecto es difícil de ser estimado usando series clínicas.

5. En la correlación fenotipo-genotipo del polimorfismo de *TMEM106B*, no se objetivó evidencia de asociación en ninguno de los modelos utilizados (genotipo, dominante y recesivo), excepto en los antecedentes familiares de demencia del fenotipo DFTvc, que fueron menos frecuentes en aquellos portadores del genotipo CC de protección; por lo que se concluye que el análisis del SNP rs1990622 no puede ser usado para diferenciar los diferentes subtipos clínicos.

6. No se han encontrado mutaciones en el gen *UBQLN2* en la población estudiada, por lo que se deduce que es una mutación rara y a tener en cuenta sólo cuando se hayan descartado las más prevalentes.

7. Estudios realizados en colaboración (Tabla 7)

   - Se ha confirmado una mutación de *TAU* y seis variantes potencialmente patogénicas de *TREM2*.
   - El estudio GWAS detecta dos loci asociados al sistema inmune y la vía lisosomal y de autofagia, como potencialmente implicados en la DFT.

Tabla 7: Estudios en colaboración

Gen	Grupo colaborador	Muestras cedidas	Mutaciones detectadas
TAU	Barcelona	14	1 (7%)
GRN	Barcelona	14	0
C9orf72	Toronto	159	6 (3.7%)
SQSTM1	Bélgica	147	0
TREM 2	Alemania	148	6 (4%)

COROLARIO

En resumen, la variabilidad fenotípica y genotípica de las DLFT representa un importante reto para el clínico, sobre todo en las fases precoces, aunque los criterios diagnósticos utilizados en la actualidad, en parte debido a la introducción de los nuevos biomarcadores (bioquímicos y de neuroimagen), permiten una clasificación fenotípica más acertada. La asociación de los marcadores genéticos al diagnóstico clínico como herramienta para conocer el sustrato molecular subyacente es fundamental para la búsqueda de nuevas terapias. En este sentido, los trabajos de esta monografía intentan abordar la importancia del estudio de estos marcadores y su correlación con la clínica de cara a un mejor conocimiento de la comunidad científica.

Es importante resaltar la dificultad, desde el punto de vista del clínico y dentro de la Sanidad pública española y catalana, el acceso a la determinación de los marcadores genéticos, que en la actualidad se han de realizar en centros de investigación o en Unidades específicas en base a convenios de colaboración, pero desconocidas para el clínico no experto en enfermedades neurodegenerativas. Probablemente un no despreciable porcentaje de casos con fenotipo DFTvc, sobre todo entre la población psiquiátrica de debut más tardío, están infradiagnosticados, no permitiendo el acceso precoz de estos pacientes a los ensayos clínicos de DFT en el presente y un tratamiento específico en el futuro. La colaboración entre las diferentes áreas clínicas se muestra aquí fundamental.

X. Referencias

Achi, Eugene Y, and Stacy A Rudnicki. 2012. "ALS and Frontotemporal Dysfunction: A Review." Neurology Research International 2012 (January): 806306. doi:10.1155/2012/806306. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3423946&tool=pmcentrez&rendertype=abstract.

Amador-Ortiz, Catalina, Wen-Lang Lin, Zeshan Ahmed, David Personett, Peter Davies, Ranjan Duara, Neill R Graff-Radford, Michael L Hutton, and Dennis W Dickson. 2007. "TDP-43 Immunoreactivity in Hippocampal Sclerosis and Alzheimer's Disease." Annals of Neurology 61 (5) (May): 435–45. doi:10.1002/ana.21154. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2677204&tool=pmcentrez&rendertype=abstract.

Appel, Stanley H, and Lewis P Rowland. 2012. "Amyotrophic Lateral Sclerosis, Frontotemporal Lobar Dementia, and p62: A Functional Convergence?" Neurology 79 (15) (October 9): 1526–7. doi:10.1212/WNL.0b013e31826e26ec. http://www.ncbi.nlm.nih.gov/pubmed/22972643.

Arai, Tetsuaki, Masato Hasegawa, Haruhiko Akiyama, Kenji Ikeda, Takashi Nonaka, Hiroshi Mori, David Mann, et al. 2006. "TDP-43 Is a Component of Ubiquitin-Positive Tau-Negative Inclusions in Frontotemporal Lobar Degeneration and Amyotrophic Lateral Sclerosis." Biochemical and Biophysical Research Communications 351 (3) (December 22): 602–11. doi:10.1016/j.bbrc.2006.10.093. http://www.ncbi.nlm.nih.gov/pubmed/17084815.

Armstrong, Melissa J, Irene Litvan, Anthony E Lang, Thomas H Bak, Kailash P Bhatia, Barbara Borroni, Adam L Boxer, et al. 2013. "Criteria for the Diagnosis of Corticobasal Degeneration." Neurology 80 (5) (January 29): 496–503. doi:10.1212/WNL.0b013e31827f0fd1. http://www.ncbi.nlm.nih.gov/pubmed/23359374.

Arvanitakis, Zoe. 2010. "Update on Frontotemporal Dementia." The Neurologist. doi:10.1097/NRL.0b013e3181b1d5c6. http://www.ncbi.nlm.nih.gov/pubmed/22277388.

Baborie, a., T. D. Griffiths, E. Jaros, P. Momeni, I. G. McKeith, D. J. Burn, G. Keir, a. J. Larner, D. M. Mann, and R. Perry. 2012. "Frontotemporal Dementia in Elderly Individuals." Archives of Neurology (April 23): 1–9. doi:10.1001/archneurol.2011.3323. http://archneur.ama-assn.org/cgi/doi/10.1001/archneurol.2011.3323.

Baker, Matt, Ian R Mackenzie, Stuart M Pickering-Brown, Jennifer Gass, Rosa Rademakers, Caroline Lindholm, Julie Snowden, et al. 2006. "Mutations in Progranulin Cause Tau-Negative Frontotemporal Dementia Linked to Chromosome 17." Nature 442 (7105) (August 24): 916–9. doi:10.1038/nature05016. http://www.ncbi.nlm.nih.gov/pubmed/16862116.

Benajiba, Lina, Isabelle Le Ber, Agnès Camuzat, Mathieu Lacoste, Catherine Thomas-Anterion, Philippe Couratier, Solenn Legallic, et al. 2009. "TARDBP Mutations in Motoneuron Disease with Frontotemporal Lobar Degeneration." Annals of Neurology 65 (4) (April): 470–3. doi:10.1002/ana.21612. http://www.ncbi.nlm.nih.gov/pubmed/19350673.

Bird, Thomas, David Knopman, John VanSwieten, Sonia Rosso, Howard Feldman, Hirotaka Tanabe, Neil Graff-Raford, Daniel Geschwind, Patrice Verpillat, and Michael Hutton. 2003. "Epidemiology and Genetics of Frontotemporal dementia/Pick's Disease." Annals of Neurology 54 Suppl 5 (January): S29–31. doi:10.1002/ana.10572. http://www.ncbi.nlm.nih.gov/pubmed/12833366.

Boeve, Bradley F, Kevin B Boylan, Neill R Graff-Radford, Mariely DeJesus-Hernandez, David S Knopman, Otto Pedraza, Prashanthi Vemuri, et al. 2012. "Characterization of Frontotemporal Dementia And/or Amyotrophic Lateral Sclerosis Associated with the GGGGCC Repeat Expansion in C9ORF72." Brain : A Journal of Neurology 135 (Pt 3) (March): 765–83. doi:10.1093/brain/aws004. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3286335&tool=pmcentrez&rendertype=abstract.

Boeve, Bradley F, and Mike Hutton. 2008. "Refining Frontotemporal Dementia with Parkinsonism Linked to Chromosome 17: Introducing FTDP-17 (MAPT) and FTDP-17 (PGRN)." Archives of Neurology 65 (4) (May 1): 460–4. doi:10.1001/archneur.65.4.460. http://archneur.jamanetwork.com/article.aspx?articleid=795385.

Cruts, Marc, Samir Kumar-Singh, and Christine Van Broeckhoven. 2006. "Progranulin Mutations in Ubiquitin-Positive Frontotemporal Dementia Linked to Chromosome 17q21." Current Alzheimer Research 3 (5) (December): 485–91. http://www.ncbi.nlm.nih.gov/pubmed/17168647.

Deerlin, Vivianna M Van, Patrick M A Sleiman, Maria Martinez-lage, Alice Chen-, Li-san Wang, Neill R Graff-radford, Dennis W Dickson, et al. 2010. "NIH Public Access" 42 (3): 234–239. doi:10.1038/ng.536. Common.

DeJesus-Hernandez, Mariely, Ian R Mackenzie, Bradley F Boeve, Adam L Boxer, Matt Baker, Nicola J Rutherford, Alexandra M Nicholson, et al. 2011. "Expanded GGGGCC Hexanucleotide Repeat in Noncoding Region of C9ORF72 Causes Chromosome 9p-Linked FTD and ALS." Neuron 72 (2) (October 20): 245–56. doi:10.1016/j.neuron.2011.09.011. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3202986&tool=pmcentrez&rendertype=abstract.

Deng, Han-Xiang, Wenjie Chen, Seong-Tshool Hong, Kym M Boycott, George H Gorrie, Nailah Siddique, Yi Yang, et al. 2011. "Mutations in UBQLN2 Cause Dominant X-Linked Juvenile and Adult-Onset ALS and ALS/dementia." Nature 477 (7363) (September 8): 211–5. doi:10.1038/nature10353. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3169705&tool=pmcentrez&rendertype=abstract.

Dreyfuss, Gideon, V Narry Kim, and Naoyuki Kataoka. 2002. "Messenger-RNA-Binding Proteins and the Messages They Carry." Nature Reviews. Molecular Cell Biology 3 (3) (March): 195–205. doi:10.1038/nrm760. http://dx.doi.org/10.1038/nrm760.

Ferrari, Raffaele, Dena G Hernandez, Michael a Nalls, Jonathan D Rohrer, Adaikalavan Ramasamy, John B J Kwok, Carol Dobson-Stone, et al. 2014. "Frontotemporal Dementia and Its Subtypes: A Genome-Wide Association Study." Lancet Neurology 13 (7) (July): 686–99. doi:10.1016/S1474-4422(14)70065-1. http://www.ncbi.nlm.nih.gov/pubmed/24943344.

Ferrer, I, I Hernández, M Boada, A Llorente, M J Rey, A Cardozo, M Ezquerra, and B Puig. 2003. "Primary Progressive Aphasia as the Initial Manifestation of Corticobasal Degeneration and Unusual Tauopathies." Acta Neuropathologica 106 (5) (November): 419–35. doi:10.1007/s00401-003-0756-4. http://www.ncbi.nlm.nih.gov/pubmed/12955398.

Ferrer, Isidro, Isabel Hernández, Berta Puig, María Jesús Rey, Mario Ezquerra, Eduardo Tolosa, and Merce Boada. 2003. "Ubiquitin-Negative Mini-Pick-like Bodies in the Dentate Gyrus in p301l Tauopathy." Journal of Alzheimer's Disease : JAD 5 (6) (December): 445–54. http://www.ncbi.nlm.nih.gov/pubmed/14757934.

Ferrer, Isidro, Gabriel Santpere, and Fred W van Leeuwen. 2008. "Argyrophilic Grain Disease." Brain : A Journal of Neurology 131 (Pt 6) (June): 1416–32. doi:10.1093/brain/awm305. http://www.ncbi.nlm.nih.gov/pubmed/18234698.

Foster, N L, K Wilhelmsen, A A Sima, M Z Jones, C J D'Amato, and S Gilman. 1997. "Frontotemporal Dementia and Parkinsonism Linked to Chromosome 17: A Consensus Conference. Conference Participants." Annals of Neurology 41 (6) (June): 706–15. doi:10.1002/ana.410410606. http://www.ncbi.nlm.nih.gov/pubmed/9189031.

Gass, Jennifer, Ashley Cannon, Ian R Mackenzie, Bradley Boeve, Matt Baker, Jennifer Adamson, Richard Crook, et al. 2006. "Mutations in Progranulin Are a Major Cause of Ubiquitin-Positive Frontotemporal Lobar Degeneration." Human Molecular Genetics 15 (20) (October 15): 2988–3001. doi:10.1093/hmg/ddl241. http://www.ncbi.nlm.nih.gov/pubmed/16950801.

Gijselinck, I, C Van Broeckhoven, and M Cruts. 2008. "Granulin Mutations Associated with Frontotemporal Lobar Degeneration and Related Disorders: An Update." Human Mutation 29 (12) (December): 1373–86. doi:10.1002/humu.20785. http://www.ncbi.nlm.nih.gov/pubmed/18543312.

Gijselinck, Ilse, Tim Van Langenhove, Julie van der Zee, Kristel Sleegers, Stéphanie Philtjens, Gernot Kleinberger, Jonathan Janssens, et al. 2012. "A C9orf72 Promoter Repeat Expansion in a Flanders-Belgian Cohort with Disorders of the Frontotemporal Lobar Degeneration-Amyotrophic Lateral Sclerosis Spectrum: A Gene Identification Study." Lancet Neurology 11 (1) (January): 54–65. doi:10.1016/S1474-4422(11)70261-7. http://www.ncbi.nlm.nih.gov/pubmed/22154785.

Gilberti, Nicola, Marinella Turla, Antonella Alberici, Valeria Bertasi, Patrizia Civelli, Silvana Archetti, Alessandro Padovani, and Barbara Borroni. 2012. "Prevalence of Frontotemporal Lobar Degeneration in an Isolated Population: The Vallecamonica Study." Neurological Sciences : Official Journal of the Italian Neurological Society and of the Italian Society of Clinical Neurophysiology 33 (4) (August): 899–904. doi:10.1007/s10072-011-0865-0. http://www.ncbi.nlm.nih.gov/pubmed/22127750.

Gislason, T B, M Sjögren, L Larsson, and I Skoog. 2003. "The Prevalence of Frontal Variant Frontotemporal Dementia and the Frontal Lobe Syndrome in a Population Based Sample of 85 Year Olds." Journal of Neurology, Neurosurgery, and Psychiatry 74 (7) (July): 867–71. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1738520&tool=pmcentrez&rendertype=abstract.

Goldman, J S, J M Farmer, E M Wood, J K Johnson, a Boxer, J Neuhaus, C Lomen-Hoerth, et al. 2005. "Comparison of Family Histories in FTLD Subtypes and Related Tauopathies." Neurology 65 (11) (December 13): 1817–9. doi:10.1212/01.wnl.0000187068.92184.63. http://www.ncbi.nlm.nih.gov/pubmed/16344531.

Gorno-Tempini, M L, A E Hillis, S Weintraub, A Kertesz, M Mendez, S F Cappa, J M Ogar, et al. 2011. "Classification of Primary Progressive Aphasia and Its Variants." Neurology 76 (11) (March 15): 1006–14. doi:10.1212/WNL.0b013e31821103e6. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3059138&tool=pmcentrez&rendertype=abstract.

Gorno-Tempini, Maria Luisa, Nina F Dronkers, Katherine P Rankin, Jennifer M Ogar, La Phengrasamy, Howard J Rosen, Julene K Johnson, Michael W Weiner, and Bruce L Miller. 2004. "Cognition and Anatomy in Three Variants of Primary Progressive Aphasia." Annals of Neurology 55 (3) (March): 335–46. doi:10.1002/ana.10825. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2362399&tool=pmcentrez&rendertype=abstract.

Grafman, J, I Litvan, and M Stark. 1995. "Neuropsychological Features of Progressive Supranuclear Palsy." Brain and Cognition 28 (3) (August): 311–20. doi:10.1006/brcg.1995.1260. http://dx.doi.org/10.1006/brcg.1995.1260.

Grossman, Murray. 2012. "The Non-Fluent/agrammatic Variant of Primary Progressive Aphasia." Lancet Neurology 11 (6) (June): 545–55. doi:10.1016/S1474-4422(12)70099-6. http://www.ncbi.nlm.nih.gov/pubmed/22608668.

Guyant-Maréchal, L, a Laquerrière, C Duyckaerts, C Dumanchin, J Bou, F Dugny, I Le Ber, T Frébourg, D Hannequin, and D Campion. 2006. "Valosin-Containing Protein Gene Mutations: Clinical and Neuropathologic Features." Neurology 67 (4) (August 22): 644–51. doi:10.1212/01.wnl.0000225184.14578.d3. http://www.ncbi.nlm.nih.gov/pubmed/16790606.

Gydesen, S., J.M. Brown, A. Brun, L. Chakrabarti, A. Gade, P. Johannsen, M. Rossor, et al. 2002. "Chromosome 3 Linked Frontotemporal Dementia (FTD-3)." Neurology 59 (10) (November 26): 1585–1594. doi:10.1212/01.WNL.0000034763.54161.1F. http://www.neurology.org/content/59/10/1585.long.

Halliday, Glenda, Eileen H Bigio, Nigel J Cairns, Manuela Neumann, Ian R a Mackenzie, and David M a Mann. 2012. "Mechanisms of Disease in Frontotemporal Lobar Degeneration: Gain of Function versus Loss of Function Effects." Acta Neuropathologica. doi:10.1007/s00401-012-1030-4. http://www.ncbi.nlm.nih.gov/pubmed/22878865.

Hernández, Isabel, Anna Espinosa, Luis Miguel Real, Jose Jorge Galán, Ana Mauleón, Maiteé Rosende Roca, Lluís Tárraga, Agustín Ruiz, and Mercè Boada. 2012. "Molecular Evaluation of Human Ubiquilin 2 Gene PXX Domain in Familial Frontotemporal Dementia Patients." Journal of Neurology 259 (11) (November): 2488–90. doi:10.1007/s00415-012-6568-5. http://www.ncbi.nlm.nih.gov/pubmed/22729385.

Hodges, John R, and Karalyn Patterson. 2007. "Semantic Dementia: A Unique Clinicopathological Syndrome." Lancet Neurology 6 (11) (November): 1004–14. doi:10.1016/S1474-4422(07)70266-1. http://www.ncbi.nlm.nih.gov/pubmed/17945154.

Holm, Ida Elisabeth, Elisabet Englund, Ian R A Mackenzie, Peter Johannsen, and Adrian M Isaacs. 2007. "A Reassessment of the Neuropathology of Frontotemporal Dementia Linked to Chromosome 3." Journal of Neuropathology and Experimental Neurology 66 (10) (October): 884–91. doi:10.1097/nen.0b013e3181567f02. http://www.ncbi.nlm.nih.gov/pubmed/17917582.

Hsiung, Ging-Yuek R, Mariely Dejesus-Hernandez, Howard H Feldman, Pheth Sengdy, Phoenix Bouchard-Kerr, Emily Dwosh, Rachel Butler, et al. 2012. "Clinical and Pathological Features of Familial Frontotemporal Dementia Caused by C9ORF72 Mutation on Chromosome 9p." Brain : A Journal of Neurology 135 (Pt 3) (March): 709–22. doi:10.1093/brain/awr354. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3286328&tool=pmcentrez&rendertype=abstract.

Hutton, M, C L Lendon, P Rizzu, M Baker, S Froelich, H Houlden, S Pickering-Brown, et al. 1998. "Association of Missense and 5'-Splice-Site Mutations in Tau with the Inherited Dementia FTDP-17." Nature 393 (6686) (July 18): 702–5. doi:10.1038/31508. http://www.ncbi.nlm.nih.gov/pubmed/9641683.

Ikejima, Chiaki, Fumihiko Yasuno, Katsuyoshi Mizukami, Megumi Sasaki, Satoshi Tanimukai, and Takashi Asada. 2009. "Prevalence and Causes of Early-Onset Dementia in Japan: A Population-Based Study." Stroke; a Journal of Cerebral Circulation 40 (8) (August 1): 2709–14. doi:10.1161/STROKEAHA.108.542308. http://stroke.ahajournals.org/content/40/8/2709.long.

Johnson, Julene K, Janine Diehl, Mario F Mendez, John Neuhaus, Jill S Shapira, Mark Forman, Dennis J Chute, et al. 2005a. "Frontotemporal Lobar Degeneration: Demographic Characteristics of 353 Patients." Archives of Neurology 62 (6) (June): 925–30. doi:10.1001/archneur.62.6.925. http://www.ncbi.nlm.nih.gov/pubmed/15956163.

———. 2005b. "Frontotemporal Lobar Degeneration: Demographic Characteristics of 353 Patients." Archives of Neurology 62 (6) (June 1): 925–30. doi:10.1001/archneur.62.6.925. http://archneur.jamanetwork.com/article.aspx?articleid=788706.

Josephs, K a, J L Whitwell, D S Knopman, B F Boeve, P Vemuri, M L Senjem, J E Parisi, et al. 2009. "Two Distinct Subtypes of Right Temporal Variant Frontotemporal Dementia." Neurology 73 (18) (November 3): 1443–50. doi:10.1212/WNL.0b013e3181bf9945. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2779005&tool=pmcentrez&rendertype=abstract.

Josephs, Keith A, John R Hodges, Julie S Snowden, Ian R Mackenzie, Manuela Neumann, David M Mann, and Dennis W Dickson. 2011. "Neuropathological Background of Phenotypical Variability in Frontotemporal Dementia." Acta Neuropathologica 122 (2) (August): 137–53. doi:10.1007/s00401-011-0839-6. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3232515&tool=pmcentrez&rendertype=abstract.

Kertesz, A, L Hudson, I R Mackenzie, and D G Munoz. 1994. "The Pathology and Nosology of Primary Progressive Aphasia." Neurology 44 (11) (November): 2065–72. http://www.ncbi.nlm.nih.gov/pubmed/7969961.

Kertesz, A, P Martinez-Lage, W Davidson, and D G Munoz. 2000. "The Corticobasal Degeneration Syndrome Overlaps Progressive Aphasia and Frontotemporal Dementia." Neurology 55 (9) (November 14): 1368–75. http://www.ncbi.nlm.nih.gov/pubmed/11087783.

Kertesz, Andrew, Mervin Blair, Paul McMonagle, and David G Munoz. 2007. "The Diagnosis and Course of Frontotemporal Dementia." Alzheimer Disease and Associated Disorders 21 (2): 155–63. doi:10.1097/WAD.0b013e31806547eb. http://www.ncbi.nlm.nih.gov/pubmed/17545742.

Kimonis, Virginia E, Sarju G Mehta, Erin C Fulchiero, Dana Thomasova, Marzia Pasquali, Kym Boycott, Edward G Neilan, et al. 2008. "Clinical Studies in Familial VCP Myopathy Associated with Paget Disease of Bone and Frontotemporal Dementia." American Journal of Medical Genetics. Part A 146A (6) (March 15): 745–57. doi:10.1002/ajmg.a.31862. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2467391&tool=pmcentrez&rendertype=abstract.

Kimonis, Virginia E, and Giles D J Watts. 2013. "Autosomal Dominant Inclusion Body Myopathy, Paget Disease of Bone, and Frontotemporal Dementia." Alzheimer Disease and Associated Disorders 19 Suppl 1: S44–7. Accessed June 5. http://www.ncbi.nlm.nih.gov/pubmed/16317258.

Kwiatkowski, T J, D A Bosco, A L Leclerc, E Tamrazian, C R Vanderburg, C Russ, A Davis, et al. 2009. "Mutations in the FUS/TLS Gene on Chromosome 16 Cause Familial Amyotrophic Lateral Sclerosis." Science (New York, N.Y.) 323 (5918) (February 27): 1205–8. doi:10.1126/science.1166066. http://www.ncbi.nlm.nih.gov/pubmed/19251627.

Lang, Christina M, Katrin Fellerer, Benjamin M Schwenk, Peer-Hendrik Kuhn, Elisabeth Kremmer, Dieter Edbauer, Anja Capell, and Christian Haass. 2012. "Membrane Orientation and Subcellular Localization of Transmembrane Protein 106B (TMEM106B), a Major Risk Factor for Frontotemporal Lobar Degeneration." The Journal of Biological Chemistry 287 (23) (June 1): 19355–65. doi:10.1074/jbc.M112.365098. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3365973&tool=pmcentrez&rendertype=abstract.

Le Ber, Isabelle, Agnès Camuzat, Didier Hannequin, Florence Pasquier, Eric Guedj, Anne Rovelet-Lecrux, Valérie Hahn-Barma, et al. 2008. "Phenotype Variability in Progranulin Mutation Carriers: A Clinical, Neuropsychological, Imaging and Genetic Study." Brain : A Journal of Neurology 131 (Pt 3) (March 1): 732–46. doi:10.1093/brain/awn012. http://brain.oxfordjournals.org/content/131/3/732.long.

Le Ber, Isabelle, Julie van der Zee, Didier Hannequin, Ilse Gijselinck, Dominique Campion, Michèle Puel, Annie Laquerrière, et al. 2007. "Progranulin Null Mutations in Both Sporadic and Familial Frontotemporal Dementia." Human Mutation 28 (9) (September): 846–55. doi:10.1002/humu.20520. http://www.ncbi.nlm.nih.gov/pubmed/17436289.

Leroy, E, R Boyer, G Auburger, B Leube, G Ulm, E Mezey, G Harta, et al. 1998. "The Ubiquitin Pathway in Parkinson's Disease." Nature 395 (6701) (October 1): 451–2. doi:10.1038/26652. http://dx.doi.org/10.1038/26652.

Litvan, I, Y Agid, D Calne, G Campbell, B Dubois, R C Duvoisin, C G Goetz, et al. 1996. "Clinical Research Criteria for the Diagnosis of Progressive Supranuclear Palsy (Steele-Richardson-Olszewski Syndrome): Report of the NINDS-SPSP International Workshop." Neurology 47 (1) (July): 1–9. http://www.ncbi.nlm.nih.gov/pubmed/8710059.

Lynch, T, M Sano, K S Marder, K L Bell, N L Foster, R F Defendini, A A Sima, C Keohane, T G Nygaard, and S Fahn. 1994. "Clinical Characteristics of a Family with Chromosome 17-Linked Disinhibition-Dementia-Parkinsonism-Amyotrophy Complex." Neurology 44 (10) (October): 1878–84. http://www.ncbi.nlm.nih.gov/pubmed/7936241.

Mackenzie, I R A, Manuela Neumann, Eileen H Bigio, Nigel J Cairns, Irina Alafuzoff, Jillian Kril, Gabor G Kovacs, et al. 2009. "Nomenclature for Neuropathologic Subtypes of Frontotemporal Lobar Degeneration: Consensus Recommendations." Acta Neuropathologica 117 (1) (January): 15–8. doi:10.1007/s00401-008-0460-5. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2710877&tool=pmcentrez&rendertype=abstract.

Mackenzie, Ian R A, Dean Foti, John Woulfe, and Trevor A Hurwitz. 2008. "Atypical Frontotemporal Lobar Degeneration with Ubiquitin-Positive, TDP-43-Negative Neuronal Inclusions." Brain : A Journal of Neurology 131 (Pt 5) (May 1): 1282–93. doi:10.1093/brain/awn061. http://brain.oxfordjournals.org/cgi/content/long/131/5/1282.

Mackenzie, Ian R a, David G Munoz, Hirofumi Kusaka, Osamu Yokota, Kenji Ishihara, Sigrun Roeber, Hans a Kretzschmar, Nigel J Cairns, and Manuela Neumann. 2011. "Distinct Pathological Subtypes of FTLD-FUS." Acta Neuropathologica 121 (2) (February): 207–18. doi:10.1007/s00401-010-0764-0. http://www.ncbi.nlm.nih.gov/pubmed/21052700.

Mackenzie, Ian R a, Manuela Neumann, Atik Baborie, Deepak M Sampathu, Daniel Du Plessis, Evelyn Jaros, Robert H Perry, John Q Trojanowski, David M a Mann, and Virginia M Y Lee. 2011. "A Harmonized Classification System for FTLD-TDP Pathology." Acta Neuropathologica 122 (1) (July): 111–3. doi:10.1007/s00401-011-0845-8. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3285143&tool=pmcentrez&rendertype=abstract.

Mahoney, Colin J, Jon Beck, Jonathan D Rohrer, Tammaryn Lashley, Kin Mok, Tim Shakespeare, Tom Yeatman, et al. 2012. "Frontotemporal Dementia with the C9ORF72 Hexanucleotide Repeat Expansion: Clinical, Neuroanatomical and Neuropathological Features." Brain : A Journal of Neurology 135 (Pt 3) (March): 736–50. doi:10.1093/brain/awr361. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3286330&tool=pmcentrez&rendertype=abstract.

Majounie, Elisa, Alan E Renton, Kin Mok, Elise Gp Dopper, Adrian Waite, Sara Rollinson, Adriano Chiò, et al. 2012. "Frequency of the C9orf72 Hexanucleotide Repeat Expansion in Patients with Amyotrophic Lateral Sclerosis and Frontotemporal Dementia: A Cross-Sectional Study." Lancet Neurology 4422 (12) (March 9): 1–8. doi:10.1016/S1474-4422(12)70043-1. http://www.ncbi.nlm.nih.gov/pubmed/22406228.

Mesulam, M M. 1982. "Slowly Progressive Aphasia without Generalized Dementia." Annals of Neurology 11 (6) (June): 592–8. doi:10.1002/ana.410110607. http://www.ncbi.nlm.nih.gov/pubmed/7114808.

Millecamps, Stéphanie, Philippe Corcia, Cécile Cazeneuve, Séverine Boillée, Danielle Seilhean, Véronique Danel-Brunaud, Nadia Vandenberghe, et al. 2012. "Mutations in UBQLN2 Are Rare in French Amyotrophic Lateral Sclerosis." Neurobiology of Aging 33 (4) (April): 839.e1–3. doi:10.1016/j.neurobiolaging.2011.11.010. http://www.ncbi.nlm.nih.gov/pubmed/22169395.

Mummery, C J, K Patterson, C J Price, J Ashburner, R S Frackowiak, and J R Hodges. 2000. "A Voxel-Based Morphometry Study of Semantic Dementia: Relationship between Temporal Lobe Atrophy and Semantic Memory." Annals of Neurology 47 (1) (January): 36–45. http://www.ncbi.nlm.nih.gov/pubmed/10632099.

Nakashima-Yasuda, Hanae, Kunihiro Uryu, John Robinson, Sharon X Xie, Howard Hurtig, John E Duda, Steven E Arnold, et al. 2007. "Co-Morbidity of TDP-43 Proteinopathy in Lewy Body Related Diseases." Acta Neuropathologica 114 (3) (September): 221–9. doi:10.1007/s00401-007-0261-2. http://www.ncbi.nlm.nih.gov/pubmed/17653732.

Neary, D, J S Snowden, L Gustafson, U Passant, D Stuss, S Black, M Freedman, et al. 1998. "Frontotemporal Lobar Degeneration: A Consensus on Clinical Diagnostic Criteria." Neurology 51 (6) (December): 1546–54. http://www.ncbi.nlm.nih.gov/pubmed/9855500.

Neumann, Manuela, Sigrun Roeber, Hans a Kretzschmar, Rosa Rademakers, Matt Baker, and Ian R a Mackenzie. 2009. "Abundant FUS-Immunoreactive Pathology in Neuronal Intermediate Filament Inclusion Disease." Acta Neuropathologica 118 (5) (November): 605–16. doi:10.1007/s00401-009-0581-5. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2864784&tool=pmcentrez&rendertype=abstract.

Neumann, Manuela, Deepak M Sampathu, Linda K Kwong, Adam C Truax, Matthew C Micsenyi, Thomas T Chou, Jennifer Bruce, et al. 2006. "Ubiquitinated TDP-43 in Frontotemporal Lobar Degeneration and Amyotrophic Lateral Sclerosis." Science (New York, N.Y.) 314 (5796) (October 6): 130–3. doi:10.1126/science.1134108. http://www.ncbi.nlm.nih.gov/pubmed/17023659.

Noble, Wendy, Diane P Hanger, Christopher C J Miller, and Simon Lovestone. 2013. "The Importance of Tau Phosphorylation for Neurodegenerative Diseases." Frontiers in Neurology 4 (July) (January): 83. doi:10.3389/fneur.2013.00083. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3696910&tool=pmcentrez&rendertype=abstract.

Paloneva BM, J., T. Autti, R. Raininko, J. Partanen, O. Salonen, M. Puranen, P. Hakola, and M. Haltia. 2001. "CNS Manifestations of Nasu-Hakola Disease: A Frontal Dementia with Bone Cysts." Neurology 56 (11) (June 12): 1552–1558. doi:10.1212/WNL.56.11.1552. http://www.neurology.org/cgi/doi/10.1212/WNL.56.11.1552.

Parkinson, N, P G Ince, M O Smith, R Highley, G Skibinski, P M Andersen, K E Morrison, et al. 2006. "ALS Phenotypes with Mutations in CHMP2B (charged Multivesicular Body Protein 2B)." Neurology 67 (6) (September 26): 1074–7. doi:10.1212/01.wnl.0000231510.89311.8b. http://www.ncbi.nlm.nih.gov/pubmed/16807408.

Pickering-Brown, Stuart M, Sara Rollinson, Daniel Du Plessis, Karen E Morrison, Anoop Varma, Anna M T Richardson, David Neary, Julie S Snowden, and David M a Mann. 2008. "Frequency and Clinical Characteristics of Progranulin Mutation Carriers in the Manchester Frontotemporal Lobar Degeneration Cohort: Comparison with Patients with MAPT and No Known Mutations." Brain : A Journal of Neurology 131 (Pt 3) (March): 721–31. doi:10.1093/brain/awm331. http://www.ncbi.nlm.nih.gov/pubmed/18192287.

Pietroboni, Anna M, Giorgio G Fumagalli, Laura Ghezzi, Chiara Fenoglio, Francesca Cortini, Maria Serpente, Claudia Cantoni, et al. 2011. "Phenotypic Heterogeneity of the GRN Asp22fs Mutation in a Large Italian Kindred." Journal of Alzheimer's Disease : JAD 24 (2) (January): 253–9. doi:10.3233/JAD-2011-101704. http://www.ncbi.nlm.nih.gov/pubmed/21258152.

Piguet, Olivier, Michael Hornberger, Eneida Mioshi, and John R Hodges. 2011. "Behavioural-Variant Frontotemporal Dementia: Diagnosis, Clinical Staging, and Management." Lancet Neurology 10 (2) (February): 162–72. doi:10.1016/S1474-4422(10)70299-4. http://www.ncbi.nlm.nih.gov/pubmed/21147039.

Poorkaj, P, T D Bird, E Wijsman, E Nemens, R M Garruto, L Anderson, A Andreadis, W C Wiederholt, M Raskind, and G D Schellenberg. 1998. "Tau Is a Candidate Gene for Chromosome 17 Frontotemporal Dementia." Annals of Neurology 43 (6) (June): 815–25. doi:10.1002/ana.410430617. http://www.ncbi.nlm.nih.gov/pubmed/9629852.

Rabinovici, Gil D, William J Jagust, Ansgar J Furst, Jennifer M Ogar, Caroline A Racine, Elizabeth C Mormino, James P O'Neil, et al. 2008. "Abeta Amyloid and Glucose Metabolism in Three Variants of Primary Progressive Aphasia." Annals of Neurology 64 (4) (October): 388–401. doi:10.1002/ana.21451. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2648510&tool=pmcentrez&rendertype=abstract.

Rascovsky, Katya, John R Hodges, David Knopman, Mario F Mendez, Joel H Kramer, John Neuhaus, John C van Swieten, et al. 2011. "Sensitivity of Revised Diagnostic Criteria for the Behavioural Variant of Frontotemporal Dementia." Brain : A Journal of Neurology 134 (Pt 9) (September): 2456–77. doi:10.1093/brain/awr179. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3170532&tool=pmcentrez&rendertype=abstract.

Ratnavalli, E., C. Brayne, K. Dawson, and J. R. Hodges. 2002. "The Prevalence of Frontotemporal Dementia." Neurology 58 (11) (June 11): 1615–1621. doi:10.1212/WNL.58.11.1615. http://www.neurology.org/content/58/11/1615.long.

Renton, Alan E, Elisa Majounie, Adrian Waite, Javier Simón-Sánchez, Sara Rollinson, J Raphael Gibbs, Jennifer C Schymick, et al. 2011. "A Hexanucleotide Repeat Expansion in C9ORF72 Is the Cause of Chromosome 9p21-Linked ALS-FTD." Neuron 72 (2) (October 20): 257–68. doi:10.1016/j.neuron.2011.09.010. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3200438&tool=pmcentrez&rendertype=abstract.

Respondek, Gesine, Sigrun Roeber, Hans Kretzschmar, Claire Troakes, Safa Al-Sarraj, Ellen Gelpi, Carles Gaig, et al. 2013. "Accuracy of the National Institute for Neurological Disorders and Stroke/Society for Progressive Supranuclear Palsy and Neuroprotection and Natural History in Parkinson Plus Syndromes Criteria for the Diagnosis of Progressive Supranuclear Palsy." Movement Disorders : Official Journal of the Movement Disorder Society 28 (4) (February 21): 504–9. doi:10.1002/mds.25327. http://www.ncbi.nlm.nih.gov/pubmed/23436751.

Roeber, Sigrun, Hansjoerg Bäzner, Michael Hennerici, Romy Porstmann, and Hans A Kretzschmar. 2006. "Neurodegeneration with Features of NIFID and ALS--Extended Clinical and Neuropathological Spectrum." Brain Pathology (Zurich, Switzerland) 16 (3) (July): 228–34. doi:10.1111/j.1750-3639.2006.00013.x. http://www.ncbi.nlm.nih.gov/pubmed/16911480.

Roeber, Sigrun, Ian R A Mackenzie, Hans A Kretzschmar, and Manuela Neumann. 2008. "TDP-43-Negative FTLD-U Is a Significant New Clinico-Pathological Subtype of FTLD." Acta Neuropathologica 116 (2) (August): 147–57. doi:10.1007/s00401-008-0395-x. http://www.ncbi.nlm.nih.gov/pubmed/18536926.

Rohrer, J D, R Guerreiro, J Vandrovcova, J Uphill, D Reiman, J Beck, a M Isaacs, et al. 2009. "The Heritability and Genetics of Frontotemporal Lobar Degeneration." Neurology 73 (18) (November 3): 1451–6. doi:10.1212/WNL.0b013e3181bf997a. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2779007&tool=pmcentrez&rendertype=abstract.

Rohrer, Jonathan D, Gerard R Ridgway, Marc Modat, Sebastien Ourselin, Simon Mead, Nick C Fox, Martin N Rossor, and Jason D Warren. 2010. "Distinct Profiles of Brain Atrophy in Frontotemporal Lobar Degeneration Caused by Progranulin and Tau Mutations." NeuroImage 53 (3) (November 15): 1070–6. doi:10.1016/j.neuroimage.2009.12.088. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2941039&tool=pmcentrez&rendertype=abstract.

Rollinson, Sara, Simon Mead, Julie Snowden, Anna Richardson, Jonathan Rohrer, Nicola Halliwell, Suzanne Usher, et al. 2011. "Frontotemporal Lobar Degeneration Genome Wide Association Study Replication Confirms a Risk Locus Shared with Amyotrophic Lateral Sclerosis." Neurobiology of Aging 32 (4) (April): 758.e1–7. doi:10.1016/j.neurobiolaging.2010.12.005. http://www.ncbi.nlm.nih.gov/pubmed/21257233.

Rosso, Sonia M, Laura Donker Kaat, Timo Baks, Marijke Joosse, Inge de Koning, Yolande Pijnenburg, Daniëlle de Jong, et al. 2003. "Frontotemporal Dementia in The Netherlands: Patient Characteristics and Prevalence Estimates from a Population-Based Study." Brain : A Journal of Neurology 126 (Pt 9) (September 1): 2016–22. doi:10.1093/brain/awg204. http://brain.oxfordjournals.org/content/126/9/2016.long.

Rosso, Sonia M, and John C van Swieten. 2002. "New Developments in Frontotemporal Dementia and Parkinsonism Linked to Chromosome 17." Current Opinion in Neurology 15 (4) (August): 423–8. http://www.ncbi.nlm.nih.gov/pubmed/12151838.

Ruiz, Agustín, Oriol Dols-Icardo, María J Bullido, Pau Pastor, Eloy Rodríguez-Rodríguez, Adolfo López de Munain, Marian M de Pancorbo, et al. 2014. "Assessing the Role of the TREM2 p.R47H Variant as a Risk Factor for Alzheimer's Disease and Frontotemporal Dementia." Neurobiology of Aging 35 (2) (February): 444.e1–4. doi:10.1016/j.neurobiolaging.2013.08.011. http://www.ncbi.nlm.nih.gov/pubmed/24041969.

Rutherford, Nicola J, Michael G Heckman, Mariely Dejesus-Hernandez, Matt C Baker, Alexandra I Soto-Ortolaza, Sruti Rayaprolu, Heather Stewart, et al. 2012. "Length of Normal Alleles of C9ORF72 GGGGCC Repeat Do Not Influence Disease Phenotype." Neurobiology of Aging 33 (12) (December): 2950.e5–7. doi:10.1016/j.neurobiolaging.2012.07.005. http://www.ncbi.nlm.nih.gov/pubmed/22840558.

Sieben, Anne, Tim Van Langenhove, Sebastiaan Engelborghs, Jean-Jacques Martin, Paul Boon, Patrick Cras, Peter-Paul De Deyn, Patrick Santens, Christine Van Broeckhoven, and Marc Cruts. 2012. "The Genetics and Neuropathology of Frontotemporal Lobar Degeneration." Acta Neuropathologica 124 (3) (September): 353–72. doi:10.1007/s00401-012-1029-x. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3422616&tool=pmcentrez&rendertype=abstract.

Skibinski, Gaia, Nicholas J Parkinson, Jeremy M Brown, Lisa Chakrabarti, Sarah L Lloyd, Holger Hummerich, Jørgen E Nielsen, et al. 2005. "Mutations in the Endosomal ESCRTIII-Complex Subunit CHMP2B in Frontotemporal Dementia." Nature Genetics 37 (8) (August): 806–8. doi:10.1038/ng1609. http://dx.doi.org/10.1038/ng1609.

Spillantini, Maria Grazia, Thomas D. Bird, and Bernardino Ghetti. 2006. "Frontotemporal Dementia and Parkinsonism Linked to Chromosome 17: A New Group of Tauopathies." Brain Pathology 8 (2) (April 5): 387–402. doi:10.1111/j.1750-3639.1998.tb00162.x. http://doi.wiley.com/10.1111/j.1750-3639.1998.tb00162.x.

Sreedharan, Jemeen, Ian P Blair, Vineeta B Tripathi, Xun Hu, Caroline Vance, Boris Rogelj, Steven Ackerley, et al. 2008. "TDP-43 Mutations in Familial and Sporadic Amyotrophic Lateral Sclerosis." Science (New York, N.Y.) 319 (5870) (March 21): 1668–72. doi:10.1126/science.1154584. http://www.ncbi.nlm.nih.gov/pubmed/18309045.

Statement, Consensus. 1994. Clinical and Neuropathological Criteria for Frontotemporal Dementia.

Strong, Michael J, Gloria M Grace, Morris Freedman, Cathy Lomen-Hoerth, Susan Woolley, Laura H Goldstein, Jennifer Murphy, et al. 2009. "Consensus Criteria for the Diagnosis of Frontotemporal Cognitive and Behavioural Syndromes in Amyotrophic Lateral Sclerosis." Amyotrophic Lateral Sclerosis : Official Publication of the World Federation of Neurology Research Group on Motor Neuron Diseases 10 (3) (June): 131–46. http://www.ncbi.nlm.nih.gov/pubmed/19462523.

Tedesco, Anna M, Francesca R Chiricozzi, Silvia Clausi, Michela Lupo, Marco Molinari, and Maria G Leggio. 2011. "The Cerebellar Cognitive Profile." Brain : A Journal of Neurology 134 (Pt 12) (December): 3672–86. doi:10.1093/brain/awr266. http://www.ncbi.nlm.nih.gov/pubmed/22036960.

Teyssou, Elisa, Takahiro Takeda, Vincent Lebon, Séverine Boillée, Brahima Doukouré, Guillaume Bataillon, Véronique Sazdovitch, et al. 2013. "Mutations in SQSTM1 Encoding p62 in Amyotrophic Lateral Sclerosis: Genetics and Neuropathology." Acta Neuropathologica 125 (4) (April): 511–22. doi:10.1007/s00401-013-1090-0. http://www.ncbi.nlm.nih.gov/pubmed/23417734.

Thelen, Mathias, Cristina Razquin, Isabel Hernández, Ana Gorostidi, Raquel Sánchez-Valle, Sara Ortega-Cubero, Steffen Wolfsgruber, et al. 2014. "Investigation of the Role of Rare TREM2 Variants in Frontotemporal Dementia Subtypes." Neurobiology of Aging (June 20). doi:10.1016/j.neurobiolaging.2014.06.018. http://www.ncbi.nlm.nih.gov/pubmed/25042114.

Turner, R S, L C Kenyon, J Q Trojanowski, N Gonatas, and M Grossman. 1996. "Clinical, Neuroimaging, and Pathologic Features of Progressive Nonfluent Aphasia." Annals of Neurology 39 (2) (February): 166–73. doi:10.1002/ana.410390205. http://www.ncbi.nlm.nih.gov/pubmed/8967747.

Urwin, Hazel, Astrid Authier, Jorgen E Nielsen, Daniel Metcalf, Caroline Powell, Kristina Froud, Denise S Malcolm, et al. 2010. "Disruption of Endocytic Trafficking in Frontotemporal Dementia with CHMP2B Mutations." Human Molecular Genetics 19 (11) (June 1): 2228–38. doi:10.1093/hmg/ddq100. http://hmg.oxfordjournals.org/content/19/11/2228.long.

Van der Zee, Julie, Ilse Gijselinck, Lubina Dillen, Tim Van Langenhove, Jessie Theuns, Sebastiaan Engelborghs, Stéphanie Philtjens, et al. 2013. "A Pan-European Study of the C9orf72 Repeat Associated with FTLD: Geographic Prevalence, Genomic Instability, and Intermediate Repeats." Human Mutation 34 (2) (February): 363–73. doi:10.1002/humu.22244. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3638346&tool=pmcentrez&rendertype=abstract.

Van der Zee, Julie, Tim Van Langenhove, Gernot Kleinberger, Kristel Sleegers, Sebastiaan Engelborghs, Rik Vandenberghe, Patrick Santens, et al. 2011. "TMEM106B Is Associated with Frontotemporal Lobar Degeneration in a Clinically Diagnosed Patient Cohort." Brain : A Journal of Neurology 134 (Pt 3) (March): 808–15. doi:10.1093/brain/awr007. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3044834&tool=pmcentrez&rendertype=abstract.

Van der Zee, Julie, Tim Van Langenhove, Gabor G Kovacs, Lubina Dillen, William Deschamps, Sebastiaan Engelborghs, Radoslav Matěj, et al. 2014. "Rare Mutations in SQSTM1 Modify Susceptibility to Frontotemporal Lobar Degeneration." Acta Neuropathologica (June 5). doi:10.1007/s00401-014-1298-7. http://www.ncbi.nlm.nih.gov/pubmed/24899140.

Van Langenhove, T, J van der Zee, K Sleegers, S Engelborghs, R Vandenberghe, I Gijselinck, M Van den Broeck, et al. 2010. "Genetic Contribution of FUS to Frontotemporal Lobar Degeneration." Neurology 74 (5) (February 2): 366–71. doi:10.1212/WNL.0b013e3181ccc732. http://www.ncbi.nlm.nih.gov/pubmed/20124201.

Vance, Caroline, Boris Rogelj, Tibor Hortobágyi, Kurt J De Vos, Agnes Lumi Nishimura, Jemeen Sreedharan, Xun Hu, et al. 2009. "Mutations in FUS, an RNA Processing Protein, Cause Familial Amyotrophic Lateral Sclerosis Type 6." Science (New York, N.Y.) 323 (5918) (February 27): 1208–11. doi:10.1126/science.1165942. http://www.ncbi.nlm.nih.gov/pubmed/19251628.

Villa, Chiara, Laura Ghezzi, Anna M Pietroboni, Chiara Fenoglio, Francesca Cortini, Maria Serpente, Claudia Cantoni, et al. 2011. "A Novel MAPT Mutation Associated with the Clinical Phenotype of Progressive Nonfluent Aphasia." Journal of Alzheimer's Disease : JAD 26 (1) (January): 19–26. doi:10.3233/JAD-2011-102124. http://www.ncbi.nlm.nih.gov/pubmed/21558644.

Watts, Giles D J, Jill Wymer, Margaret J Kovach, Sarju G Mehta, Steven Mumm, Daniel Darvish, Alan Pestronk, Michael P Whyte, and Virginia E Kimonis. 2004. "Inclusion Body Myopathy Associated with Paget Disease of Bone and Frontotemporal Dementia Is Caused by Mutant Valosin-Containing Protein." Nature Genetics 36 (4) (April): 377–81. doi:10.1038/ng1332. http://dx.doi.org/10.1038/ng1332.

Weihl, C C. 2011. "Valosin Containing Protein Associated Frontotemporal Lobar Degeneration: Clinical Presentation, Pathologic Features and Pathogenesis." Current Alzheimer Research 8 (3) (May): 252–60. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3246398&tool=pmcentrez&rendertype=abstract.

Whitwell, Jennifer L, and Keith a Josephs. 2012. "Neuroimaging in Frontotemporal Lobar Degeneration-Predicting Molecular Pathology." Nature Reviews. Neurology 8 (3) (January): 131–42. doi:10.1038/nrneurol.2012.7. http://www.ncbi.nlm.nih.gov/pubmed/22290573.

Whitwell, Jennifer L, Scott a Przybelski, Stephen D Weigand, Robert J Ivnik, Prashanthi Vemuri, Jeffrey L Gunter, Matthew L Senjem, et al. 2009. "Distinct Anatomical Subtypes of the Behavioural Variant of Frontotemporal Dementia: A Cluster Analysis Study." Brain : A Journal of Neurology 132 (Pt 11) (November): 2932–46. doi:10.1093/brain/awp232. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2768663&tool=pmcentrez&rendertype=abstract.

Whitwell, Jennifer L, Stephen D Weigand, Bradley F Boeve, Matthew L Senjem, Jeffrey L Gunter, Mariely Dejesus-Hernandez, Nicola J Rutherford, et al. 2012. "Neuroimaging Signatures of Frontotemporal Dementia Genetics: C9ORF72, Tau, Progranulin and Sporadics." Brain : A Journal of Neurology 135 (Pt 3) (March): 794–806. doi:10.1093/brain/aws001. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3286334&tool=pmcentrez&rendertype=abstract.

Wood, Elisabeth M, Dana Falcone, Eunran Suh, David J Irwin, Alice S Chen-Plotkin, Edward B Lee, Sharon X Xie, Vivianna M Van Deerlin, and Murray Grossman. 2013. "Development and Validation of Pedigree Classification Criteria for Frontotemporal Lobar Degeneration." JAMA Neurology 70 (11) (November): 1411–7. doi:10.1001/jamaneurol.2013.3956. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3906581&tool=pmcentrez&rendertype=abstract.

Xi, Zhengrui, Lorne Zinman, Yakov Grinberg, Danielle Moreno, Christine Sato, Juan M Bilbao, Mahdi Ghani, et al. 2012. "Investigation of C9orf72 in 4 Neurodegenerative Disorders." Archives of Neurology (September 10): 1–8. doi:10.1001/archneurol.2012.2016. http://www.ncbi.nlm.nih.gov/pubmed/22964832.

Yokota, Osamu, Kuniaki Tsuchiya, Seishi Terada, Hideki Ishizu, Hirotake Uchikado, Manabu Ikeda, Kiyomitsu Oyanagi, et al. 2008. "Basophilic Inclusion Body Disease and Neuronal Intermediate Filament Inclusion Disease: A Comparative Clinicopathological Study." Acta Neuropathologica 115 (5) (May): 561–75. doi:10.1007/s00401-007-0329-z. http://www.ncbi.nlm.nih.gov/pubmed/18080129.

Yu, Chang-en, Thomas D Bird, Lynn M Bekris, Thomas J Montine, James B Leverenz, Ellen Steinbart, Nichole M Galloway, et al. 2010. "The Spectrum of Mutations in Progranulin :" 67 (2): 161–170. doi:10.1001/archneurol.2009.328.The.

Zhao, Lihong, Christine Rosales, Kevin Seburn, David Ron, and Susan L Ackerman. 2010. "Alteration of the Unfolded Protein Response Modifies Neurodegeneration in a Mouse Model of Marinesco-Sjögren Syndrome." Human Molecular Genetics 19 (1) (January 1): 25–35. doi:10.1093/hmg/ddp464. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2792147&tool=pmcentrez&rendertype=abstract.

XI. Anexo

OTRAS PUBLICACIONES RELACIONADAS CON LA SERIE CLINICA OBJETO DE TESIS

1. ***Molecular evaluation of human ubiquilin 2 gene PXX domain in familial fronto-temporal dementia patients***. **Hernández I**, Espinosa A, Real LM, Galán JJ, Mauleón A, Roca MR, Tárraga L, Ruiz A, Boada M. *J Neurol.* 2012 Nov;259(11):2488-90. doi: 10.1007/s00415-012-6568-5. Epub 2012 Jun 24.

   IF: 3.578 (2012) Q1

2. ***Investigation of c9orf72 in 4 neurodegenerative disorders***. Xi Z, Zinman L, Grinberg Y, Moreno D, Sato C, Bilbao JM, Ghani M, **Hernández I**, Ruiz A, Boada M, Morón FJ, Lang AE, Marras C, Bruni A, Colao R, Maletta RG, Puccio G, Rainero I, Pinessi L, Galimberti D, Morrison KE, Moorby C, Stockton JD, Masellis M, Black SE, Hazrati LN, Liang Y, van Haersma de With J, Fornazzari L, Villagra R, Rojas-Garcia R, Clarimón J, Mayeux R, Robertson J, St George-Hyslop P, Rogaeva E.*Arch Neurol.* 2012 Dec;69(12):1583-90.

   IF: 7.584 (2012) Q1

3. ***Expansion mutation in C9ORF72 does not influence plasma progranulin levels in frontotemporal dementia.*** Dols-Icardo O, Suárez-Calvet M, **Hernández I,** Amer G, Antón-Aguirre S, Alcolea D, Fortea J, Boada M, Tárraga L, Blesa R, Lleó A, Clarimón J. *Neurobiol Aging*. 2012 Aug; 33(8):1851.e17-9.doi: 10.1016/j.neurobiolaging.2012.03.005. Epub 2012 Apr 11.

   IF: 6.166 (2012) Q1

4. ***Plasma phosphorylated TDP-43 levels are elevated in patients with frontotemporal dementia carrying a C9orf72 repeat expansion or a GRN mutation.*** Suárez-Calvet M, Dols-Icardo O, Lladó A, Sánchez-Valle R, **Hernández I**, Amer G, Antón-Aguirre S, Alcolea D, Fortea J, Ferrer I, van der Zee J, Dillen L, Van Broeckhoven C, Molinuevo JL, Blesa R, Clarimón J, Lleó A. *J Neurol Neurosurg Psychiatry*. 2014 Jun; 85(6):684-91. doi: 10.1136/jnnp-2013-305972. Epub 2013 Dec 4.

   IF: 4.764 (2013) Q1

5. ***Characterization of the repeat expansion size in C9orf72 in amyotrophic lateral sclerosis and frontotemporal dementia.*** Dols-Icardo O, García-Redondo A, Rojas-García R, Sánchez-Valle R, Noguera A, Gómez-Tortosa E, Pastor P, **Hernández I**, Esteban-Pérez J, Suárez-Calvet M, Antón-Aguirre S, Amer G, Ortega-Cubero S, Blesa R, Fortea J, Alcolea D, Capdevila A, Antonell A, Lladó A, Muñoz-Blanco JL, Mora JS, Galán-Dávila L, Rodríguez De Rivera FJ, Lleó A, Clarimón J. *Hum Mol Genet*. 2014 Feb. 1; 23(3):749-54. doi: 10.1093/hmg/ddt460. Epub 2013 Sep 20.

   I.F: 7.692 (2014) Q1

6. ***Assessing the role of the TREM2 p.R47H variant as a risk factor for Alzheimer's disease and frontotemporal dementia.*** Ruiz A, Dols-Icardo O, Bullido MJ, Pastor P, Rodríguez-Rodríguez E, López de Munain A, de Pancorbo MM, Pérez-Tur J, Alvarez V, Antonell A, López-Arrieta J, **Hernández I**, Tárraga L, Boada M, Lleó A, Blesa R, Frank-García A, Sastre I, Razquin C, Ortega-Cubero S, Lorenzo E, Sánchez-Juan P, Combarros O, Moreno F, Gorostidi A, Elcoroaristizabal X, Baquero M, Coto E, Sánchez-Valle R, Clarimón J; Dementia genetic Spanish consortium (DEGESCO). *Neurobiol Aging*. 2014 Feb; 35(2):444.e1-4. doi: 10.1016/j.neurobiolaging.2013.08.011. Epub 2013 Sep 13.

   IF: 6.166 (2014) Q1

7. ***Frontotemporal dementia and its subtypes: a genome-wide association study.*** Ferrari R, Hernandez DG, Nalls MA, Rohrer JD, Ramasamy A, Kwok JB, Dobson-Stone C, Brooks WS, Schofield PR, Halliday GM, Hodges JR, Piguet O, Bartley L, Thompson E, Haan E, **Hernández I**, Ruiz A, Boada M, Borroni B, Padovani A, Cruchaga C, Cairns NJ, Benussi L, Binetti G, Ghidoni R, Forloni G, Galimberti D, Fenoglio C, Serpente M, Scarpini E, Clarimón J, Lleó A, Blesa R, Waldö ML, Nilsson K, Nilsson C, Mackenzie IR, Hsiung GY, Mann DM, Grafman J, Morris CM, Attems J, Griffiths TD, McKeith IG, Thomas AJ, Pietrini P, Huey ED, Wassermann EM, Baborie A, Jaros E, Tierney MC, Pastor P, Razquin C, Ortega-Cubero S, Alonso E, Perneczky R, Diehl-Schmid J, Alexopoulos P, Kurz A, Rainero I, Rubino E, Pinessi L, Rogaeva E, St George-Hyslop P, Rossi G, Tagliavini F, Giaccone G, Rowe JB, Schlachetzki JC, Uphill J, Collinge J, Mead S, Danek A, Van Deerlin VM, Grossman M, Trojanowski JQ, van der Zee J, Deschamps W, Van Langenhove T, Cruts M, Van Broeckhoven C, Cappa SF, Le Ber I, Hannequin D, Golfier V, Vercelletto M, Brice A, Nacmias B, Sorbi S, Bagnoli S, Pia-

ceri I, Nielsen JE, Hjermind LE, Riemenschneider M, Mayhaus M, Ibach B, Gasparoni G, Pichler S, Gu W, Rossor MN, Fox NC, Warren JD, Spillantini MG, Morris HR, Rizzu P, Heutink P, Snowden JS, Rollinson S, Richardson A, Gerhard A, Bruni AC, Maletta R, Frangipane F, Cupidi C, Bernardi L, Anfossi M, Gallo M, Conidi ME, Smirne N, Rademakers R, Baker M, Dickson DW, Graff-Radford NR, Petersen RC, Knopman D, Josephs KA, Boeve BF, Parisi JE, Seeley WW, Miller BL, Karydas AM, Rosen H, van Swieten JC, Dopper EG, Seelaar H, Pijnenburg YA, Scheltens P, Logroscino G, Capozzo R, Novelli V, Puca AA, Franceschi M, Postiglione A, Milan G, Sorrentino P, Kristiansen M, Chiang HH, Graff C, Pasquier F, Rollin A, Deramecourt V, Lebert F, Kapogiannis D, Ferrucci L, Pickering-Brown S, Singleton AB, Hardy J, Momeni P.

Lancet Neurol. 2014 Jul;13(7):686-99. doi: 10.1016/S1474-4422(14)70065-1.

IP: 23.917 (2014) Q1

8. ***Rare mutations in SQSTM1 modify susceptibility to frontotemporal lobar degeneration.*** van der Zee J1, Van Langenhove T, Kovacs GG, Dillen L, Deschamps W, Engelborghs S, Matěj R, Vandenbulcke M, Sieben A, Dermaut B, Smets K, Van Damme P, Merlin C, Laureys A, Van Den Broeck M, Mattheijssens M, Peeters K, Benussi L, Binetti G, Ghidoni R, Borroni B, Padovani A, Archetti S, Pastor P,Razquin C, Ortega-Cubero S, **Hernández I**, Boada M, Ruiz A, de Mendonça A, Miltenberger-Miltényi G, do Couto FS, Sorbi S, Nacmias B, Bagnoli S, Graff C,Chiang HH, Thonberg H, Perneczky R, Diehl-Schmid J, Alexopoulos P, Frisoni GB, Bonvicini C, Synofzik M, Maetzler W, Vom Hagen JM, Schöls L, Haack TB,Strom TM, Prokisch H, Dols-Icardo O, Clarimón J, Lleó A, Santana I, Almeida MR, Santiago B, Heneka MT, Jessen F, Ramirez A, Sanchez-Valle R, Llado A,Gelpi E, Sarafov S, Tournev I, Jordanova A, Parobkova E, Fabrizi GM, Testi S, Salmon E, Ströbel T, Santens P, Robberecht W, De Jonghe P, Martin JJ, Cras P,Vandenberghe R, De Deyn PP, Cruts M, Sleegers K, Van Broeckhoven C.

Acta Neuropathol. 2014 Jun 5. . [Epub ahead of print]

IF: 9.734. (2014) Q1

9. ***Investigation of the role of rare TREM2 variants in frontotemporal dementia subtypes.*** Thelen M, Razquin C, **Hernández I**, Gorostidi A, Sánchez-Valle R, Ortega-Cubero S, Wolfsgruber S, Drichel D, Fliessbach K, Duenkel T, Damian M, Heilmann S,

Slotosch A, Lennarz M, Seijo-Martínez M, Rene R, Kornhuber J, Peters O, Luckhaus C, Jahn H, Hüll M, Rüther E, Wiltfang J, Lorenzo E, Gascon J, Lleó A, Lladó A, Camp-delacreu J, Moreno F, Ahmadzadehfar H; Dementia Genetics Spanish Consortium (DEGESCO), Fortea J, Indakoetxea B, Heneka MT, Wetter A, Pastor MA, Riverol M, Becker T, Frölich L, Tárraga L, Boada M, Wagner M, Jessen F, Maier W, Clarimón J, López de Munain A, Ruiz A, Pastor P, Ramirez A.

Neurobiol Aging. 2014 Jun 20. pii: S0197-4580(14)00437-0. doi: 10.1016/j.neurobio-laging.2014.06.018. [Epub ahead of print]

IF: 6.166 (2014) Q1

10. ***Ubicuitin-negative mini-pick-like bodies in the dentate gyrus in p301l tauopathy.*** Ferrer I, **Hernández I**, Puig B, Rey MJ, Ezquerra M, Tolosa E, Boada M. J Alzheimer's Dis. 2003 Dec; 5(6):445-54.

11. ***Primary progressive aphasia as the initial manifestation of corticobasal degeneration and unusual tauopathies.*** Ferrer I, **Hernández I**, Boada M, Llorente A, Rey MJ, Cardozo A, Ezquerra M, Puig B. Acta Neuropathol. 2003 Nov;106(5):419-35. Epub 2003 Aug 29.

IF: 6.49 (2003) Q1

J Neurol
DOI 10.1007/s00415-012-6568-5

LETTER TO THE EDITORS

Molecular evaluation of human Ubiquilin 2 gene PXX domain in familial frontotemporal dementia patients

Isabel Hernández · Anna Espinosa · Luis Miguel Real · Jose Jorge Galán · Ana Mauleón · Maiteé Rosende Roca · Lluís Tárraga · Agustín Ruiz · Mercè Boada

Received: 28 March 2012 / Revised: 16 May 2012 / Accepted: 18 May 2012

Dear Sirs,

Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) phenotypes appear in the same pedigrees [1] and can occur in the same individuals [2] (FTDALS, OMIM 105550). In fact about 2–3 % of ALS patients develop dementia [3]. Consequently, in some individuals, there appears to be a common genetic etiology for both diseases. Genetic variation identified in *GRN, FUS, TDP-43*, and more recently, in *C9ORF72* and *UBQLN2* genes has been associated to familial ALS (FALS, OMIN 104105), familial FTD (OMIM 600274) and/or FTDALS consistent with the notion that both disorders may share genetic components and, probably, functional pathogenic mechanisms [4–8].

UBQLN2 gene was recently identified by Deng et al. [7] as a novel dominant but not fully penetrant X-linked FTDALS gene. Specifically, five segregating mutations in four different proline residues within the PXX repeat domain of *UBQLN2* gene were identified in families afflicted with ALS/dementia. Importantly, the authors also found a correlation of hippocampal UBQLN2 pathology with dementia in ALS cases with or without *UBQLN2* mutations, suggesting that *UBQLN2* gene could be involved in ALS-related dementia, even without *UBQLN2* mutations [7].

UBQLN2 gene encodes an ubiquitin-like protein (ubiquilin) that shares a high degree of similarity with related proteins in multiple eukaryotic organisms. Specifically, ubiquilins contain an N-terminal ubiquitin-like domain and a C-terminal ubiquitin-associated domain; therefore, UBQLN2 could be functionally linked to ubiquitination enzymatic processes. Functional analysis showed that mutations in UBQLN2 lead to an impairment of protein degradation [7]. Of note, UBQLN2 protein has also been shown to bind the Stch protein which is also involved in aggresome malfunction [9]. The role of ubiquitination in neurodegeneration is well established in different human neurodegenerative disorders such Parkinson's disease or the Marinesco-Sjögren syndrome [10, 11]. Furthermore, it has been suggested that there is a link between Alzheimer's disease and ubiquitins [12].

The purpose of the present study is to further explore the role of *UBQLN2* mutations in familial FTD [13]. We examined 77 FTD index patients with a positive family history of dementia who had complete clinical evaluations. Importantly, we maintained FTD patients with observed male to male transmission (which makes a dominant X-linked transmission impossible) as negative controls of this study ($n = 12$). For the purpose of this study, we examined subjects with FTD and its subtypes (behavioral variant FTD (bvFTD), semantic dementia, progressive non-fluent aphasia), according to Neary et al. [14], as well as patients within the FTD spectrum, such as with those with combined phenotype of FTD and ALS, corticobasal syndrome or progressive supranuclear palsy (Table 1). All individuals agreed to participate in the study and blood samples were obtained after informed consent from

Electronic supplementary material The online version of this article (doi:10.1007/s00415-012-6568-5) contains supplementary material, which is available to authorized users.

I. Hernández · A. Espinosa · A. Mauleón · M. R. Roca · L. Tárraga · A. Ruiz (✉) · M. Boada
Memory Clinic of Fundació ACE, Institut Català de Neurociències Aplicades, Marquès de Sentmenat 57, 08029 Barcelona, Spain
e-mail: aruiz@fundacioace.com

L. M. Real · J. J. Galán
Department of Structural Genomics, Neocodex, Sevilla, Spain

Published online: 24 June 2012

subjects and/or their legal representatives according to GIPSY protocol, approved by our referral ethics committee in the Hospital Clinic I Provincial (Barcelona, Spain) and conforms to the World Medical Association's Declaration of Helsinki. DNA extraction from frozen blood was performed automatically according to standard procedures using the Magnapure DNA isolation system (Roche Diagnostics, Mannheim, Germany). To perform PCRs, we prepared aliquots of DNA at a concentration of 10 ng/ul. The rest of the stock was cryopreserved at −20 °C.

We employed automated DNA sequencing methods to scan the specific *UBQLN2* genomic region. The designed primer pairs to amplify this region were Fw: 5′ GTGCTG GGAACCGCTATAGG and Rv: 5′ ATTGCGTTGAG CTGTTCCAG. The PCR reactions were carried out in a final volume of 20 μL containing: 20 ng of genomic DNA, 0.25 μM each amplification primer, 62.5 μM of each dNTP, 1 M betaine (Sigma-Aldrich, Madrid, Spain), 0.5 μL of DMSO (Sigma-Aldrich), 2 μL of 10X KAPA Taq Buffer A (Kapa Biosystem, MA, USA) and 0.5 UI Taq DNA Polymerase (Kapa Biosystem). Cycling conditions were: an initial denaturation step of 95 °C for 4 min, followed by 35 cycles of 95 °C for 1 min, 61 °C for 1 min, and 72 °C for 1 min, and a final step of 72 °C for 4 min. All PCRs were performed in a Biometra thermal cycler (Biometra Tpersonal, Göttingen, Germany). After electrophoresis in 1.5 % agarose gel, the PCR products were purified using the Nucleo-Spin Gel and PCR clean-up kit (Macherey–Nagel, Düren, Germany) according to the manufacturer's instructions. Sequencing reactions were performed using the CEQ Dye Terminator Cycle Sequencing Quit Start Kit (Beckman-Coulter, Inc) and the Rv primer according to the manufacturer's instructions. Fluorograms were analyzed on CEQTM 8000 Genetic Analysis System following the manufacturer's instructions.

Reference Genomic sequence containing PXX domain of *UBQLN2* gene was identified using tools at UCSC Genome Bioinformatics server (http://genome.ucsc.edu/). We selected a 112 base pair (bp) containing 12 consecutive PXX repeats for mutation analysis (Supplementary Figure 1). Selected DNA fragments were manually inspected using Chromas software (ver 1.43, ©Conor McCarthy, Brisbane, Australia). To conduct identity, similarity and gap analyses, sequences were automatically evaluated using two different computed-based software (EMBOSS-water and Clustalw2) freely available at EBI-EMBL web site.

The manual and automated inspections of the PXX domain of *UBQLN2* gene (77 independent reads) did not find any evidence of PXX mutations in our patients. It is possible that we could have had a potential false negative in this study. However, we believe that this is less likely since we performed current gold standard methodology for mutation detection, which is the automated DNA sequencing [15], and because we have carefully controlled for any potential sequence alteration or suspicious fluorescence peak in the electropherograms at least twice, both times using independent PCR reactions. Furthermore, automated alignment analyses indicated that most base pairs analyzed within the PXX domain of *UBQLN2* gene have been safely resolved using our molecular method with a high confidence (overall identity >97 %, overall similarity 99.04 %, observed gaps <0.23 %). On the other hand, multiple sequence alignment revealed that when the noise appeared in the electropherograms it was usually concentrated in proline codons of PXX domain (Supplementary Figure 1). This technical observation is not unusual because CpGs and hairpin regions are generally concentrating sequence compression and noise [16]. Of note, noisy peaks could complicate sequencing interpretation of critical codons and mutation researchers have to be aware of it.

We failed to identify any causative mutations within the PXX domain of *UBQLN* gene in familial FTD patients of Spanish origin. Nevertheless, these negative results do not invalidate previous findings in FTDALS families and do not rule out the existence of other variants in another *UBQLN* gene domain. However, our preliminary inspection of non PXX sequences and previous results obtained in familial ALS (no mutations in 130 FALS cases) in French FALS patients [17] suggested that *UBQLN2* PXX domain germline mutations are scarce in familial FTD or ALS.

Table 1 Observed clinical phenotypes in familial FTD patients included in this study

Phenotype	Acronym	*N*	%
FTD behavior variant	FTDbv	47	61
Semantic dementia	SD	11	14.3
Progressive non fluent aphasia	PNFA	8	10.4
FTD plus motor neuron disease	FTD-MND	3	3.9
Corticobasal syndrome	CBS	4	5.2
Progressive supranuclear palsy	PSP	2	2.6
Mixed phenotype	–	2	2.6

Acknowledgments We would like to thank patients who participated in this project. We are indebted to Trinitat Port-Carbó and her family who are supporting Fundació ACE research programs.

Conflicts of interest None.

References

1. Pinsky L, Finlayson MH, Libman I, Scott BH (1975) Familial amyotrophic lateral sclerosis with dementia: a second Canadian family. Clin Genet 7:186–191
2. Robertson EE (1953) Progressive bulbar paralysis showing heredofamilial incidence and intellectual impairment. AMA Arch Neurol Psychiatry 69:196–207

3. Fecto F, Siddique T (2011) SIGMAR1 mutations, genetic heterogeneity at the chromosome 9p locus, and the expanding etiological diversity of amyotrophic lateral sclerosis. Ann Neurol 70:867–870
4. Ito D, Suzuki N (2011) Conjoint pathologic cascades mediated by ALS/FTLD-U linked RNA-binding proteins TDP-43 and FUS. Neurology 77:1636–1643
5. DeJesus-Hernandez M, Mackenzie IR, Boeve BF, Boxer AL, Baker M, Rutherford NJ, Nicholson AM, Finch NA, Flynn H, Adamson J et al (2011) Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. Neuron 72:245–256
6. Renton AE, Majounie E, Waite A, Simon-Sanchez J, Rollinson S, Gibbs JR, Schymick JC, Laaksovirta H, van Swieten JC, Myllykangas L et al (2011) A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. Neuron 72:257–268
7. Deng HX, Chen W, Hong ST, Boycott KM, Gorrie GH, Siddique N, Yang Y, Fecto F, Shi Y, Zhai H et al (2011) Mutations in UBQLN2 cause dominant X-linked juvenile and adult-onset ALS and ALS/dementia. Nature 477:211–215
8. Sleegers K, Brouwers N, Maurer-Stroh S, van Es MA, Van Damme P, van Vught PW, van der Zee J, Serneels S, De Pooter T, Van den Broeck M et al (2008) Progranulin genetic variability contributes to amyotrophic lateral sclerosis. Neurology 71:253–259
9. Kent WJ, Sugnet CW, Furey TS, Roskin KM, Pringle TH, Zahler AM, Haussler D (2002) The human genome browser at UCSC. Genome Res 12:996–1006
10. Zhao L, Rosales C, Seburn K, Ron D, Ackerman SL (2010) Alteration of the unfolded protein response modifies neurodegeneration in a mouse model of Marinesco-Sjogren syndrome. Hum Mol Genet 19:25–35
11. Leroy E, Boyer R, Auburger G, Leube B, Ulm G, Mezey E, Harta G, Brownstein MJ, Jonnalagada S, Chernova T et al (1998) The ubiquitin pathway in Parkinson's disease. Nature 395:451–452
12. Slifer MA, Martin ER, Haines JL, Pericak-Vance MA (2005) The ubiquilin 1 gene and Alzheimer's disease. N Engl J Med 352:2752–2753 author reply 2752-2753
13. Daoud H, Rouleau GA (2011) A role for ubiquilin 2 mutations in neurodegeneration. Nat Rev Neurol 7:599–600
14. Neary D, Snowden JS, Gustafson L, Passant U, Stuss D, Black S, Freedman M, Kertesz A, Robert PH, Albert M et al (1998) Frontotemporal lobar degeneration: a consensus on clinical diagnostic criteria. Neurology 51:1546–1554
15. Bellosillo B, Tusquets I (2006) Pitfalls and caveats in BRCA sequencing. Ultrastruct Pathol 30:229–235
16. Yamakawa H, Nakajima D, Ohara O (1996) Identification of sequence motifs causing band compressions on human cDNA sequencing. DNA Res 3:81–86
17. Millecamps S, Corcia P, Cazeneuve C, Boillee S, Seilhean D, Danel-Brunaud V, Vandenberghe N, Pradat PF, Le Forestier N, Lacomblez L et al (2011) Mutations in UBQLN2 are rare in French amyotrophic lateral sclerosis. Neurobiol Aging 33(4): 839.e1–839.e3

ONLINE FIRST

Investigation of *C9orf72* in 4 Neurodegenerative Disorders

Zhengrui Xi, PhD; Lorne Zinman, MD; Yakov Grinberg, PhD; Danielle Moreno, BSc; Christine Sato, MSc; Juan M. Bilbao, MD; Mahdi Ghani, MD; Isabel Hernández, MD; Agustín Ruiz, MD, PhD; Mercè Boada, MD, PhD; Francisco J. Morón, PhD; Anthony E. Lang, MD; Connie Marras, MD, PhD; Amalia Bruni, MD; Rosanna Colao, MD; Raffaele G. Maletta, MD; Gianfranco Puccio, MD; Innocenzo Rainero, MD, PhD; Lorenzo Pinessi, MD; Daniela Galimberti, PhD; Karen E. Morrison, PhD; Catriona Moorby, BSc; Joanne D. Stockton, BSc; Mario Masellis, MD; Sandra E. Black, MD; Lili-Naz Hazrati, MD; Yan Liang, MD; Jan van Haersma de With, BSc; Luis Fornazzari, MD; Roque Villagra, MD; Ricardo Rojas-Garcia, MD, PhD; Jordi Clarimón, PhD; Richard Mayeux, MD; Janice Robertson, PhD; Peter St George-Hyslop, MD, FRCP(C); Ekaterina Rogaeva, PhD

Objective: To estimate the allele frequency of *C9orf72* (G_4C_2) repeats in amyotrophic lateral sclerosis (ALS), frontotemporal lobar degeneration (FTLD), Alzheimer disease (AD), and Parkinson disease (PD).

Design: The number of repeats was estimated by a 2-step genotyping strategy. For expansion carriers, we sequenced the repeat flanking regions and obtained *APOE* genotypes and *MAPT* H1/H2 haplotypes.

Setting: Hospitals specializing in neurodegenerative disorders.

Subjects: We analyzed 520 patients with FTLD, 389 patients with ALS, 424 patients with AD, 289 patients with PD, 602 controls, 18 families, and 29 patients with PD with the *LRRK2* G2019S mutation.

Main Outcome Measure: The expansion frequency.

Results: Based on a prior cutoff (>30 repeats), the expansion was detected in 9.3% of patients with ALS, 5.2% of patients with FTLD, and 0.7% of patients with PD but not in controls or patients with AD. It was significantly associated with family history of ALS or FTLD and age at onset of FTLD. Phenotype variation (ALS vs FTLD) was not associated with *MAPT*, *APOE*, or variability in the repeat flanking regions. Two patients with PD were carriers of 39 and 32 repeats with questionable pathological significance, since the 39-repeat allele does not segregate with PD. No expansion or intermediate alleles (20-29 repeats) were found among the G2019S carriers and AD cases with TAR DNA-binding protein 43–positive inclusions. Surprisingly, the frequency of the 10-repeat allele was marginally increased in all 4 neurodegenerative diseases compared with controls, indicating the presence of an unknown risk variation in the *C9orf72* locus.

Conclusions: The C9orf72 expansion is a common cause of ALS and FTLD, but not of AD or PD. Our study raises concern about a reliable cutoff for the pathological repeat number, which is important in the utility of genetic screening.

Arch Neurol. Published online September 10, 2012. doi:10.1001/archneurol.2012.2016

AMYOTROPHIC LATERAL SCLEROSIS (ALS) and frontotemporal lobar degeneration (FTLD) are fatal neurodegenerative syndromes that belong to the same clinicopathological spectrum.[1,2] Frontotemporal lobar degeneration is a primary dementia characterized by early behavioral, language, and extrapyramidal changes, while symptoms of ALS are the result of the degeneration of motor neurons. Both syndromes may occur within the same family or even the same patient.

Previously, linkage analyses revealed a 3.7-Mb region on 9p21 associated with familial ALS/FTLD,[3-10] and genome-wide association studies suggested a major risk factor in the same locus for sporadic ALS and FTLD.[11-15] Recently, 2 research groups independently explained this locus by a pathological noncoding hexanucleotide $(G_4C_2)_{30\text{-}1600}$ repeat expansion in the chromosome 9 open reading frame 72 (*C9orf72*) gene of unknown function.[16,17] Based on the allele frequencies in cases vs controls, the first studies suggested that expansions with more than 30 repeats should be considered pathological, while alleles with less than 20 repeats are wild type.[16] However, a reliable cutoff for the pathological alleles remains to be established by

Author Affiliations are listed at the end of this article.

additional studies (eg, segregation, neuropathological, or functional studies). Furthermore, the contribution of intermediate-size alleles (20-29 repeats) to disease pathology has not yet been evaluated.

The expansion is the most frequent cause of ALS and FTLD identified to date. In the Finnish population, 46% of patients with familial ALS, 21% of patients with sporadic ALS, and 29% of patients with sporadic FTLD have the expansion.[16] DeJesus-Hernandez et al[17] reported the expansion in 24% of patients with familial ALS, 4% of patients with sporadic ALS, and 12% of patients with familial FTLD. In the Flanders-Belgian cohort, the mutation was observed in 47% of patients with familial ALS, 5% of patients with sporadic ALS, and 16% of patients with familial FTLD.[18] The pathological mechanism associated with the expansion is currently unknown except that the expansion leads to a 50% reduction of *C9orf72* messenger RNA expression,[17,18] and the brain pathology in mutation carriers is associated with possibly toxic nuclear RNA foci, as well as TAR DNA-binding protein 43 (TDP-43) and p62 inclusions.[17,19] The clinical phenotype appears to be highly heterogeneous in reported mutation carriers.[20]

Following the discovery of this novel mutation, many questions are yet to be addressed. What is the expansion frequency in other ALS/FTLD cohorts? Are there any clinical features that can discriminate between patients with and without the *C9orf72* expansion? What is a reliable cutoff for the pathological repeat number? What is the role of alleles with intermediate repeat sizes or variability in the region flanking the G_4C_2 repeat? Could the expansion account for other neurodegenerative diseases, such as Alzheimer disease (AD) or Parkinson disease (PD)? These questions were investigated by the current study. The expansion frequency was estimated in a comprehensive case-control sample set consisting of 2224 individuals (patients with FTLD, ALS, AD, and PD and healthy controls). To our knowledge, this is the first case-control study using a 2-step genotyping strategy that allowed for the analysis of genotype information for the alleles with less than 50 repeats. Clinical data were analyzed to understand the phenotype spectrum observed in mutation carriers.

METHODS

HUMAN SAMPLES

Informed consent was obtained from all participants in accordance with the respective ethical review boards. Sample characteristics are presented in **Table 1**. All study participants were either European (from Italy, Spain, and the United Kingdom) or North American (white individuals mainly of North European origin) recruited from hospitals specializing in neurodegenerative disorders. The investigated unrelated individuals included 520 patients with FTLD, 389 patients with ALS, 424 patients with AD, and 289 patients with PD and 602 neurologically healthy controls (>65 years). Patients were diagnosed using established clinical criteria.[21-24] Cases with known pathological mutations were excluded from the study. However, a previously described data set of 29 Canadian patients with PD carrying the common *LRRK2* G2019S mutation[25] was analyzed separately for the presence of an expansion or intermediate allele in *C9orf72* as a potential modifier of age at onset of parkinsonism. In addition, all available family members of 18 extended pedigrees were genotyped for the G_4C_2 repeats (6 FTLD, 9 ALS, 1 PD, and 2 AD families).

Table 1. Sample Characteristics, Including Expansion Carriers Identified in Each Cohort

Cohort	All Samples, No. (%)	No. of Expansion Carriers[a]	Frequency of Expansion, %
ALS			
Age at onset, y, mean (SD)	57.6 (12.3)		
Female	149 (38.3)		
Total	389	36	9.3
Familial	47	18	38.3
FTLD			
Age at onset, y, mean (SD)	65.4 (10.1)		
Female	258 (49.6)		
Total	520	27	5.2
Familial	211	22	10.4
AD			
Age at onset, y, mean (SD)	72.1 (9.4)		
Female	264 (62.3)		
Total	424	...	...
Familial	167	...	...
PD			
Age at onset, y, mean (SD)	52.6 (13.0)		
Female	92 (31.8)		
Total	289	2	0.7
Familial	116	1	0.9
Controls			
Age, y, mean (SD)	70.2 (9.5)		
Female	357 (59.3)		
Total	602	...	...

Abbreviations: AD, Alzheimer disease; ALS, amyotrophic lateral sclerosis; ellipses, not detected/not applicable; FTLD, frontotemporal lobar degeneration; PD, Parkinson disease

[a] The allele was counted as an expansion allele when the number of repeats was more than 30.

GENOTYPING ASSAYS

Genomic DNA was isolated from blood using a QIAGEN kit. To detect the size of the *C9orf72* alleles within the normal to intermediate range of G_4C_2 repeats (detection limit is 50 repeats) and the presence of large expansions, a 2-step genotyping strategy was used as previously described[17] (eFigure 1, http://www.joygrafika.com/projects/University_of_Toronto). Briefly, in the first step, DNA samples (10 ng/polymerase chain reaction [PCR]) were amplified using primers near the repeat region (5′-FAM-caaggagggaaacaaccgcagcc and 5′-gcaggcaccgcaaccgcag). The fragment-length analysis was performed on an ABI PRISM 3100 DNA analyzer and visualized by Genotyper software version 2.5 (Applied Biosystems). Since expanded alleles are not amplifiable with this set of primers, expansion carriers appear to be homozygous for a normal repeat allele (in addition to true homozygotes). The number of repeats was calculated based on the fragment size (eg, 129 base pairs [bp] represents 2 repeats, which was confirmed by sequencing 7 samples [PCR primers: 5′-cgtcatcgcacatagaaaaca and 5′-ggagacagctcgggtactga]). Since published sequencing analysis demonstrated that the G_4C_2 repeats are uninterrupted,[17] the number of repeats for each allele was calculated

using the following formula: (amplicon length – 117)/6. Samples scored as homozygous were included in the second step to detect large expansions (>50 repeats) by a repeat-primed PCR. DNA samples (100 ng/PCR) were amplified as described previously using 3 primers (MRX-F: FAM-tgtaaaacgacggccagtcaaggagggaaacaaccgcagcc, MRX-M13R: caggaaacagctatgacc, and MRX-R1: caggaaacagctatgaccgggcccgccccgaccacgccccggccccggccccgg),[17] except the primer ratio was modified to increase PCR efficiency (MRX-F/MRX-M13R/MRX-R1 = 5/5/1). Data were analyzed using GeneScan software version 3.1 (Applied Biosystems).

To search for sequence variability in regions flanking the G_4C_2 repeat, 2 PCR products were sequenced in 50 patients (44 expansion carriers and 6 noncarriers) (eFigure 1B). The 5′ flanking region was amplified with primers 5′-ccctaccagggtttgcagt and 5′-cgactcctgagttccagagc (616 bp). The 3′ flanking region was amplified with primers 5′-tgcggttgcggtgcctgc and 5′-gaatggggagcacaccgacttgc (625 bp). The *APOE* polymorphism defining the ε2 to ε4 alleles and the 238-bp insertion/deletion in intron 9 of *MAPT* defining the H1/H2 haplotypes was genotyped as described previously in all patients with FTLD and ALS with a pathological expansion in *C9orf72*, as well as in their family members.[26]

STATISTICAL ANALYSES

Differences in sample characteristics (eg, sex, age at onset, and familial history between cases and controls) were analyzed using the χ^2 test, Fisher exact test, or independent-samples *t* test as appropriate. Allele frequencies within the normal to intermediate range of repeats were calculated after excluding patients who carry the pathological expanded allele, defined as a repeat number more than 30 as previously suggested.[16]

The association between disease and alleles with less than 30 repeats was assessed using CLUMP software that is based on Monte Carlo tests for the evaluation of highly polymorphic loci.[27] Empirical *P* values for T1 through T4 analyses were obtained after 2000 simulations. Further analyses to obtain *P* values for each allele were also carried out as follows: allele counts from cases and controls were tested for significance using the χ^2 test after combining rare alleles. When any cell in the contingency table had an expected value less than 5, the corresponding allele was pooled with the neighboring allele (this process was repeated until no cell had an expected value <5). Each allele group was compared in turn with the rest of the alleles pooled together to calculate the χ^2 statistic. The pooling enabled the calculation of odds ratios and 95% CIs.[28] All the statistical analyses were done using SPSS (version 20; IBM SPSS). Statistical significance was taken to be $P < .05$ (Bonferroni correction for multiple testing was applied).

RESULTS

We used a previously suggested cutoff (>30 repeats) to distinguish the pathogenic expansion from the normal allele.[16] None of the patients in our series were homozygous for the expansion allele, since all samples were successfully amplified in the first step. Samples homozygous in the first step (33%) were evaluated for the large expansion (>50 repeats). Based on the electropherogram with sawtooth peaks (eFigure 1), 65 unrelated patients were identified to be expansion carriers: 9.3% of patients with ALS (36 of 389), 5.2% of patients with FTLD (27 of 520), and 0.7% of patients with PD (2 of 289), but no expansions were detected in 424 patients with AD and 602 controls (Table 1).

Details of the clinical data for expansion carriers vs noncarriers are presented in **Table 2**. In addition, 14 case reports on expansion carriers are available in the online-only material. The expansion allele was significantly associated with family history for both ALS and FTLD ($P < .001$). The frequency of the expansion was 10.5% in patients with familial FTLD and 38.3% in patients with familial ALS. The average age at onset of FTLD was 6 years younger in expansion carriers than those without ($P = .003$). The disease subcategory of FTLD with motor neuron disease was significantly enriched in expansion carriers ($P < .001$). However, there was no significant association with any evaluated clinical characteristic of ALS.

In expansion carriers, we did not observe an intermediate or pathological number of repeats for the second allele (2-11 repeats). Also, sequencing analysis of 44 expansion carriers and 6 noncarriers did not reveal variability in the regions immediately flanking the G_4C_2 repeat that could be responsible for repeat instability, including a short tandem repeat $(CGG)_8$ located 294 bp downstream of the G_4C_2 repeat (eFigure 1B). Hence, it is unlikely that the number of repeats of the second allele or polymorphisms in the flanking region contribute significantly to the disease phenotype. In addition, the *MAPT* H1/H2 and *APOE* genotypes were examined in 63 expansion carriers (36 patients with ALS and 27 patients with FTLD) and their family members, since both genes are well-known risk factors for several neurodegenerative disorders including FTLD. However, statistical analysis did not reveal a significant link between these genes and disease presentation (ALS vs FTLD) (eTable 1 and eFigure 2).

For the segregation analysis, we genotyped all available family members of probands with the expansion (6 FTLD and 9 ALS families) and detected 30 additional mutation carriers. The expansion allele showed perfect cosegregation with disease, and for those expansion carriers who were asymptomatic (mean [SD] age, 46.3 [10.7] years; range, 24-64 years), follow-up is ongoing (**Table 3**, **Figure 1**A, and eFigure 2A and B). Within the pedigrees, there was no evidence of instability in the repeat size for the normal alleles (Figure 1A and eFigure 2). The method used in the study did not allow the same question to be addressed for the expanded allele, and the DNA quality/quantity was not sufficient to conduct Southern blotting to estimate the size of the expanded allele. However, in the 2 families with expansion carriers in 2 generations, a younger age at onset in the subsequent generation was observed (48 vs 74 years in ALS15 pedigree and 45-47 years vs 60s in the FTLD TOR73 pedigree), indicating genetic anticipation (eFigure 2A).

Two patients with PD (without signs of ALS or dementia) were categorized as expansion carriers (39 and 32 repeats). Family history of PD was known only for 1 patient; however, the 39-repeat allele does not segregate with PD, since the affected sibling did not inherit it (Figure 1B). The role of intermediate alleles (20-29 repeats) in neurodegenerative diseases is currently unknown. The clinical characteristics of the patients with PD with the expansion and intermediate alleles are presented in eTable 2. Intermediate alleles were observed

Table 2. Comparison of the Clinical Statistics Between Expansion Carriers and Noncarriers

Cohort	No. (%)		*P* Value [a]	OR [b] (95% CI)
	Noncarriers	Expansion Carriers		
ALS Cases				
No. of ALS cases	353	36		
Age at onset, y, mean (SD)	57.6 (12.6)	57.8 (9.1)	.92	. . .
Female	134 (38.0)	15 (41.7)	.68	. . .
Familial cases	29 (8.2)	18 (50.0)	2.31×10^{-9}	11.2 (5.2-23.8)
Cases with FTLD	41 (11.6)	4 (11.1)	.93	. . .
Site of onset				
Limb	235 (66.6)	24 (66.7)	.99	. . .
Bulbar	93 (26.3)	10 (28.7)	.85	. . .
Mixed	7 (2.0)	. . .	. . .	. . .
Unknown	18 (5.1)	2 (5.6)	. . .	. . .
FTLD Cases				
No. of FTLD cases	493	27		
Age at onset, y, mean (SD)	65.7 (10.1)	59.6 (7.6)	.003	. . .
Female	248 (50.5)	10 (37.0)	.17	. . .
Familial cases	188 (38.1)	22 (81.5)	7.8×10^{-6}	7.1 (2.7-19.2)
Diagnosis subcategory				
bvFTD	218 (44.2)	11 (40.7)	.72	. . .
FTLD unspecified	131 (26.6)	8 (29.6)	.73	. . .
SD	60 (12.2)	. . .	. . .	. . .
PNFA	59 (12.0)	. . .	. . .	. . .
FTLD-MND	20 (4.1)	8 (29.6)	2.8×10^{-5}	10.0 [c] (3.9-25.5)
Other	5 (1.0)	. . .	. . .	. . .

Abbreviations: ALS, amyotrophic lateral sclerosis; bvFTD, behavioral variant of frontotemporal dementia; ellipses, not detected/not applicable; FTLD, frontotemporal lobar degeneration; FTLD-MND, frontotemporal lobar degeneration with motor neuron disease; OR, odds ratio; PNFA, progressive nonfluent aphasia; SD, semantic dementia.

[a] A *t* test was used to compare the continuous variables. Categorical variables were compared by Pearson χ^2 test or Fisher exact test (when expected value <5).

[b] The OR was calculated only for variables showing $P < .05$.

[c] The OR was obtained by comparing FTLD-MND with the rest of the subcategories.

Table 3. Expansion Carriers Identified in the ALS and FTLD Pedigrees

Status	Sample Size		
	All	Expansion Carriers	Noncarriers
6 FTLD pedigrees	48	20	28
FTLD	8	8	. . .
FTLD-MND	1	1	. . .
bvFTD	2	2	. . .
Relatives	30	9	21
Spouses	7	. . .	7
9 ALS pedigrees	34	25	9
ALS	7	7	. . .
ALS-FTLD	2	2	. . .
FTLD	1	1	. . .
Relatives	24	15	9

Abbreviations: ALS, amyotrophic lateral sclerosis; bvFTD, behavioral variant of frontotemporal dementia; ellipses, not detected; FTLD, frontotemporal lobar degeneration; FTLD-MND, frontotemporal lobar degeneration with motor neuron disease.

only in 4 PD cases, 2 of which had parkinsonism followed by severe dementia. We also assessed if the presence of intermediate alleles could influence the age at onset of parkinsonism in *LRRK2* G2019S mutation carriers (onset between age 40-80 years). However, none of the 29 *LRRK2* mutation carriers had an expanded or intermediate repeat allele (<11 repeats).

Furthermore, our results do not suggest that the intermediate *C9orf72* alleles significantly contribute to AD risk, since the frequency of such alleles was similar between patients with AD and controls (about 1%), and the alleles with 23 and 21 repeats did not segregate with AD in 2 families (eFigure 2C). Importantly, 15 of the 31 autopsied AD cases had TDP-43 inclusions (a frequent copathology in AD); however, no intermediate allele carriers were found among these cases. This is of note because ALS/FTLD caused by the expansion is associated with TDP-43 brain pathology.[17,20,29,30] Hence, the TDP-43 pathology in AD could not be explained by an increased number of repeats in *C9orf72*.

Finally, a case-control association analysis was carried out using Monte Carlo tests. All carriers of alleles with more than 30 repeats were excluded from this analysis to avoid association due to the pathological expansion. Analyses T1 and T2 revealed association between normal repeat alleles (<30) with FTLD and ALS (eTable 4). Analysis T3 showed that the 10-repeat allele was significantly associated with FTLD. To get statistics for each allele (eg, *P* value or odds ratio), we assessed the distribution of normal alleles in 4 disease groups vs controls (**Figure 2** and eTable 3). In each group, the most frequent were the 2-, 5-, and 8-repeat alleles, which account for about 75% of all observed alleles. A marginal protective effect of the 5-repeat allele was detected in

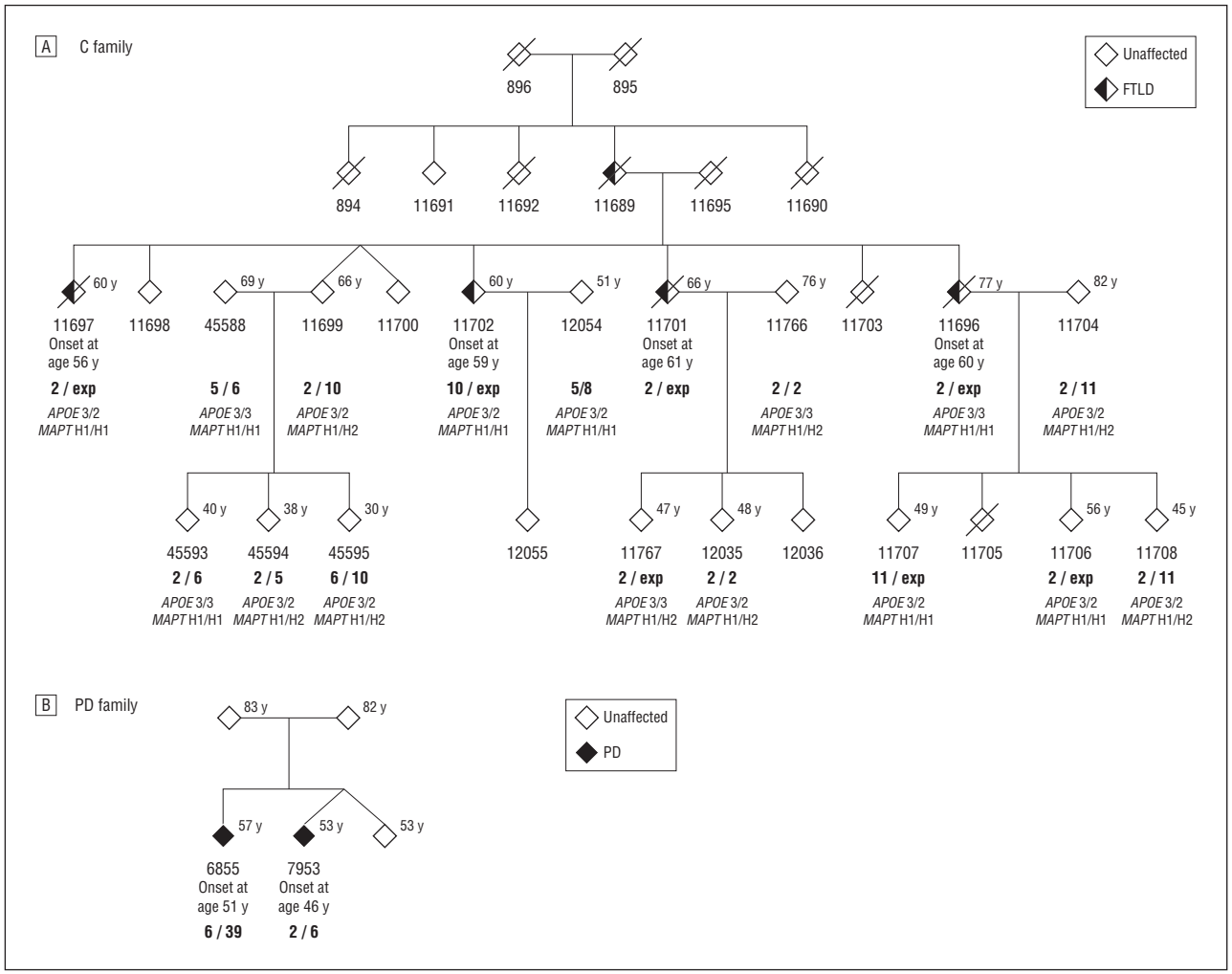


Figure 1. Families with the G_4C_2 repeat expansion. A, Frontotemporal lobar degeneration (FTLD) pedigree. B, Parkinson disease (PD) pedigree. Individual genotypes are shown beneath the corresponding diamond, including "at-risk" currently asymptomatic individuals. Arabic numbers indicate the repeat units (exp indicates expansion). The age at the time of examination is shown in the upper right corner. Age at onset is indicated for patients below the ID number. Sex of the family members is masked to protect privacy.

FTLD, ALS, and PD, as well as of the 11-repeat allele in ALS and AD (nominal $P < .05$). The frequency of the 10-repeat allele was marginally increased in all 4 neurodegenerative diseases vs the control group (nominal $P < .05$). The risk associated with this allele remained significant in the FTLD data set even after Bonferroni correction ($P = .03$; odds ratio, 2.14) (eTable 3).

COMMENT

Our findings further support the expansion in *C9orf72* as a common cause of ALS and FTLD, but not AD or PD. In contrast to the 1-step genotyping used in published case-control studies of *C9orf72*, 2-step genotyping allowed us to obtain the exact genotype for each individual (except >50 repeats). No homozygous expansion carriers were found in the current study, suggesting that such a genotype could be lethal. A heterozygous expansion (>30 repeats) was detected in 9.3% of patients with ALS and 5.2% of patients with FTLD and was significantly more frequent in cases with a family history of ALS or FTLD. The frequency was doubled in our familial FTLD cases (10.4%), which is similar to that reported by DeJesus-Hernandez et al[17] (11.7%).

We did not detect significant association between the presence of the expansion and clinical characteristics of patients with ALS, while in the FTLD series, the expansion was associated with a younger age at onset and the FTLD with motor neuron disease phenotype. These findings are in line with previous observations.[18,31] Notably, none of our FTLD cases with the expansion were diagnosed with primary progressive aphasia, which is similar to the Mayo clinic cohort[32] and different from the Manchester cohort wherein 4 primary progressive aphasia cases had the expansion.[31] Wide clinical variability in expansion carriers has been previously described (eg, age at onset and disease duration),[20,33] and future characterization of such data in a large cohort of carriers could generate a clinically useful algorithm to prioritize patients for mutation analysis of *C9orf72*.[34]

In our study, a variable phenotype in expansion carriers (ALS vs FTLD) was not associated with the *APOE* alleles or the extended *MAPT* H1/H2 haplotypes. However, only an evaluation of a large data set could reach a

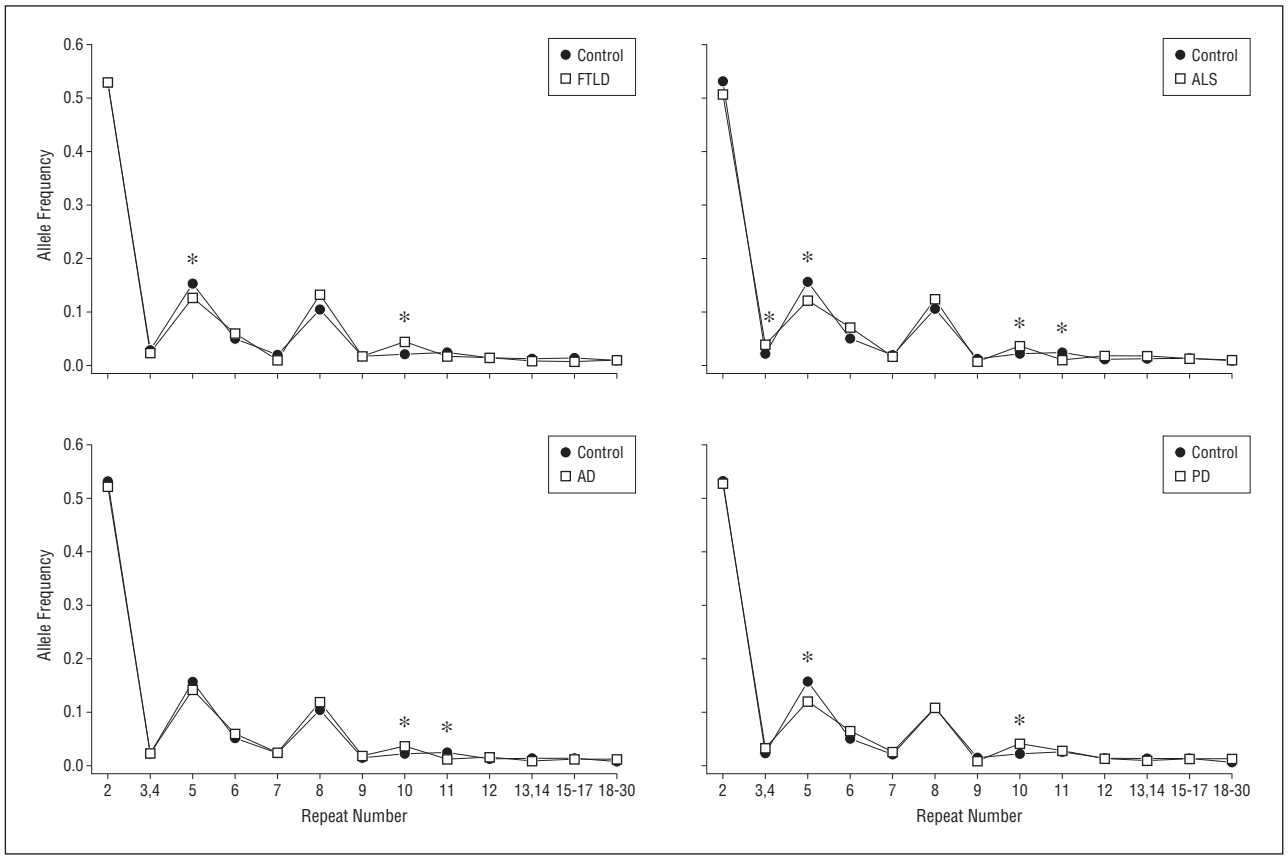


Figure 2. Comparison of the allele frequencies (repeats <30) between controls and cases in each disease cohort (AD indicates Alzheimer disease; ALS, amyotrophic lateral sclerosis; FTLD, frontotemporal lobar degeneration; and PD, Parkinson disease). Asterisks indicate a significant *P* value (nominal $P < .05$). In comparison with controls, there were 2 significant differences observed for the allele frequencies for the patients with FTLD, 4 for the patients with ALS, 2 for the patients with AD, and 2 for the patients with PD. A similar pattern was observed for all 4 diseases. The 5-repeat allele was significantly lower in FTLD, ALS, and PD than in controls and the 10-repeat allele in disease was significantly higher in all 4 disease groups than in controls. Expansion carriers were excluded from this analysis: controls, n = 602; FTLD, n = 488; ALS, n = 353; AD, n = 424; and PD, n = 287.

solid conclusion about *APOE* and *MAPT* as phenotype modifiers. It is also possible that *C9orf72* itself is responsible for the modifying effect because of coding sequence variations, repeat size, and/or repeat instability. We demonstrated that the repeat size of the second allele in expansion carriers was within the normal range (2-11 repeats) and unlikely to contribute to the variability in disease phenotype. In addition, our sequencing analysis did not reveal variations in regions flanking the G_4C_2 repeat that could be responsible for repeat instability or the variable phenotype of expansion carriers. Future studies should assess intronic and coding variations in the entire *C9orf72* locus as potential phenotype modifiers.

Instability of the G_4C_2 repeat region was suggested to be a possible mechanism for the occurrence of the expansion.[16] However, a founder effect is more likely to be responsible for the incidence of the mutation, since most carriers harbor a common haplotype.[17,29] Despite the method's limitation (the size of large expansions could not be determined), we estimated the stability of 2 to 30 repeats in the pedigrees. The number of repeats was stable across generations.

Given the clinical/pathological overlap between neurodegenerative diseases,[35-38] our study determined whether the expansion plays a role in AD or PD. Importantly, the PD samples were previously collected to generate a data set enriched in genetic predisposition to PD and mainly consisted of cases with either an early age at onset (mean [SD], 52.6 [13.04] years) and/or family history of PD (40%).[39] However, the frequency of expansion alleles in our PD data set was unremarkable (0.7%). Only 2 patients (without symptoms of ALS or dementia) were carriers of 32 and 39 repeats, which is much smaller than the expansion estimated by Southern blot (700-1600 repeats),[17] and segregation analysis did not support the pathogenic nature of the 39-repeat allele. This raises concern about a reliable cutoff for a pathological repeat number, which is important in the utility of genetic screening in patient care. Likely, a higher cutoff or establishing a gray zone will be proposed in the future based on an increasing number of observations from case-control and neuropathological studies.[40,41] Among our ALS and FTLD mutation carriers, only 2 had an allele with questionable pathological significance (<40 repeats). The connection between the repeat number and pathological significance has to be carefully investigated. It is possible that the pathological cutoff is disease dependent (eg, ALS vs FTLD) and could be modulated by individual genetic background. Furthermore, future studies have to test the possibility of somatic instability of the repeat region (the disease-related tissues, such as the spinal cord, could have

larger expansions than blood cells from the same individual).

Our results did not suggest that the expansion or intermediate alleles are associated with AD. In contrast, there was a report of 6 expansion carriers in a familial AD cohort (<1%), 4 of whom were from the same family.[42] However, autopsy indicated that 3 carriers actually had amnestic FTLD. Whether the remaining carriers were also clinically misdiagnosed as having AD remains to be seen. In addition to typical AD pathology, 15 of our patients with AD had TDP-43 inclusions, which are known to be associated with the brain pathology of the expansion[17,20,29,30]; however, all of these patients with AD have genotypes within the normal range (2-12 repeats).

Our case-control studies also assessed the frequency of alleles within the normal range (<30 repeats) and observed a trend toward an association between the 10-repeat allele and risk for all 4 disorders (odds ratio, 1.72-2.14). It is tempting to speculate that this allele is in linkage disequilibrium with an unknown *C9orf72* risk variation. Further genetic work has to validate this observation, including follow-up case-control studies and sequencing of 10-repeat carriers.

Accepted for Publication: May 30, 2012.
Published Online: September 10, 2012. doi:10.1001/archneurol.2012.2016
Author Affiliations: Tanz Centre for Research in Neurodegenerative Diseases (Drs Xi, Grinberg, Ghani, Hazrati, Liang, Robertson, St George-Hyslop, and Rogaeva, Mss Moreno and Sato, and Mr van Haersma de With), Division of Neurology, Department of Medicine (Drs Lang, Marras, Masellis, Black, St George-Hyslop, and Rogaeva), and Department of Psychiatry, St. Michael's Hospital (Dr Fornazzari), University of Toronto, Sunnybrook Health Sciences Centre (Drs Zinman, Bilbao, Masellis, and Black), Morton and Gloria Shulman Movement Disorders Clinic and the Edmond J. Safra Program in Parkinson's Disease, Toronto Western Hospital (Drs Lang and Marras), and LC Campbell Cognitive Neurology Research Unit, Sunnybrook Research Institute (Drs Masellis and Black), Toronto, Ontario, Canada; Memory Clinic of Fundació ACE, Institut Català de Neurociències Aplicades (Drs Hernández, Ruiz, and Boada), Hospital Universitari Vall d'Hebron–Institut de Recerca, Universitat Autònoma de Barcelona (VHIR-UAB) (Dr Boada), and Neurology Department, Hospital de la Santa Creu i Sant Pau, Universitat Autònoma de Barcelona (Drs Rojas-Garcia and Clarimón), Barcelona, Department of Structural Genomics, Neocodex, Seville (Dr Morón), and Center for Networker Biomedical Research in Neurodegenerative Diseases (CIBERNED), Madrid (Drs Rojas-Garcia and Clarimón), Spain; Regional Neurogenetic Centre, Lamezia Terme, Azienda Sanitaria Provinciale Catanzaro (Drs Bruni, Colao, Maletta, and Puccio), and Neurology II, Department of Neuroscience, University of Torino, Turin (Drs Rainero and Pinessi), and Department of Neurological Sciences, University of Milan, Centro Dino Ferrari, Fondazione Cà Granda, IRCCS Ospedale Maggiore Policlinico, Milan (Dr Galimberti), Italy; School of Clinical and Experimental Medicine, College of Medical and Dental Sciences, University of Birmingham, Birmingham (Dr Morrison and Mss Moorby and Stockton), and Cambridge Institute for Medical Research and the Department of Clinical Neurosciences, University of Cambridge, Cambridge (Dr St George-Hyslop), England; Salvador Hospital, University of Chile, Santiago (Dr Villagra); and The Taub Institute for Research on Alzheimer's Disease and the Aging Brain, The Gertrude H. Sergievsky Center, Departments of Neurology, Psychiatry, and Medicine, College of Physicians and Surgeons, Columbia University, New York, New York (Dr Mayeux).
Correspondence: Ekaterina Rogaeva, PhD, Tanz Centre for Neurodegenerative Diseases, 6 Queen's Park Crescent West, Toronto, ON M5S 3H2, Canada (ekaterina.rogaeva@utoronto.ca) or Peter St George-Hyslop, MD, FRCP(C) (p.hyslop@utoronto.ca).
Author Contributions: *Study concept and design:* Xi, St George-Hyslop, and Rogaeva. *Acquisition of data:* Xi, Zinman, Grinberg, Moreno, Sato, Bilbao, Ghani, Hernández, Ruiz, Boada, Morón, Lang, Marras, Bruni, Colao, Maletta, Puccio, Rainero, Pinessi, Galimberti, Morrison, Moorby, Stockton, Masellis, Black, Hazrati, Liang, van Haersma de With, Fornazzari, Villagra, Rojas-Garcia, Clarimón, Mayeux, Robertson, and Rogaeva. *Analysis and interpretation of data:* Xi, Grinberg, Marras, Masellis, and Rogaeva. *Drafting of the manuscript:* Xi and Rogaeva. *Critical revision of the manuscript for important intellectual content:* Xi, Zinman, Grinberg, Moreno, Sato, Bilbao, Ghani, Hernández, Ruiz, Boada, Morón, Lang, Marras, Bruni, Colao, Maletta, Puccio, Rainero, Pinessi, Galimberti, Morrison, Moorby, Stockton, Masellis, Black, Hazrati, Liang, van Haersma de With, Fornazzari, Villagra, Rojas-Garcia, Clarimón, Mayeux, Robertson, and St George-Hyslop. *Statistical analysis:* Xi. *Obtained funding:* Zinman, Grinberg, Bilbao, van Haersma de With, Mayeux, Robertson, St George-Hyslop, and Rogaeva. *Administrative, technical, and material support:* Xi, Zinman, Moreno, Sato, Ghani, Hernández, Ruiz, Boada, Morón, Bruni, Colao, Maletta, Puccio, Rainero, Pinessi, Galimberti, Morrison, Moorby, Stockton, Black, Hazrati, Liang, Fornazzari, Rojas-Garcia, Clarimón, and Rogaeva. *Study supervision:* Morrison, St George-Hyslop, and Rogaeva.
Financial Disclosure: Dr Masellis has received speaker honoraria from Novartis and EMD Serono Inc; serves as an associate editor for *Current Pharmacogenomics and Personalized Medicine*; receives publishing royalties from Henry Stewart Talks; has served as a consultant for BioScape Medical Imaging CRO; and receives research support from the Canadian Institutes of Health Research, Parkinson Society Canada, an Early Researcher Award from the Ministry of Economic Development and Innovation of Ontario, Teva Pharmaceutical Industries Ltd, and the Department of Medicine, Sunnybrook Health Sciences Centre.
Funding/Support: This work was supported by grants from the Ontario Research Fund, the Weston Foundation (Drs Rogaeva and St George-Hyslop), the Howard Hughes Medical Institute, The Wellcome Trust and Medical Research Council, the Alzheimer Society of Ontario (Dr St George-Hyslop), the Canadian Institutes of Health Research (Drs St George-Hyslop, Rogaeva and Black), a Center of Excellence grant from the National Parkinson

Foundation (Drs Marras and Lang), and the James Hunter Family ALS Initiative (Drs Robertson and Zinman).

Online-Only Material: The eTables, eFigures, and case reports are available at http://www.joygrafika.com/projects/University_of_Toronto.

REFERENCES

1. Fecto F, Siddique T. Making connections: pathology and genetics link amyotrophic lateral sclerosis with frontotemporal lobe dementia. *J Mol Neurosci*. 2011; 45(3):663-675.
2. Lillo P, Hodges JR. Frontotemporal dementia and motor neurone disease: overlapping clinic-pathological disorders. *J Clin Neurosci*. 2009;16(9):1131-1135.
3. Boxer AL, Mackenzie IR, Boeve BF, et al. Clinical, neuroimaging and neuropathological features of a new chromosome 9p-linked FTD-ALS family. *J Neurol Neurosurg Psychiatry*. 2011;82(2):196-203.
4. Gijselinck I, Engelborghs S, Maes G, et al. Identification of 2 loci at chromosomes 9 and 14 in a multiplex family with frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Arch Neurol*. 2010;67(5):606-616.
5. Le Ber I, Camuzat A, Berger E, et al; French Research Network on FTD/FTD-MND. Chromosome 9p-linked families with frontotemporal dementia associated with motor neuron disease. *Neurology*. 2009;72(19):1669-1676.
6. Luty AA, Kwok JB, Thompson EM, et al. Pedigree with frontotemporal lobar degeneration–motor neuron disease and Tar DNA binding protein-43 positive neuropathology: genetic linkage to chromosome 9. *BMC Neurol*. 2008;8:32.
7. Morita M, Al-Chalabi A, Andersen PM, et al. A locus on chromosome 9p confers susceptibility to ALS and frontotemporal dementia. *Neurology*. 2006;66(6): 839-844.
8. Valdmanis PN, Dupre N, Bouchard JP, et al. Three families with amyotrophic lateral sclerosis and frontotemporal dementia with evidence of linkage to chromosome 9p [published correction appears in *Arch Neurol*. 2007;64(6):909]. *Arch Neurol*. 2007;64(2):240-245.
9. Vance C, Al-Chalabi A, Ruddy D, et al. Familial amyotrophic lateral sclerosis with frontotemporal dementia is linked to a locus on chromosome 9p13.2-21.3. *Brain*. 2006;129(pt 4):868-876.
10. Pearson JP, Williams NM, Majounie E, et al. Familial frontotemporal dementia with amyotrophic lateral sclerosis and a shared haplotype on chromosome 9p. *J Neurol*. 2011;258(4):647-655.
11. Laaksovirta H, Peuralinna T, Schymick JC, et al. Chromosome 9p21 in amyotrophic lateral sclerosis in Finland: a genome-wide association study. *Lancet Neurol*. 2010;9(10):978-985.
12. Shatunov A, Mok K, Newhouse S, et al. Chromosome 9p21 in sporadic amyotrophic lateral sclerosis in the UK and seven other countries: a genome-wide association study. *Lancet Neurol*. 2010;9(10):986-994.
13. van Es MA, Veldink JH, Saris CG, et al. Genome-wide association study identifies 19p13.3 (UNC13A) and 9p21.2 as susceptibility loci for sporadic amyotrophic lateral sclerosis. *Nat Genet*. 2009;41(10):1083-1087.
14. Van Deerlin VM, Sleiman PM, Martinez-Lage M, et al. Common variants at 7p21 are associated with frontotemporal lobar degeneration with TDP-43 inclusions. *Nat Genet*. 2010;42(3):234-239.
15. Rollinson S, Mead S, Snowden J, et al. Frontotemporal lobar degeneration genome wide association study replication confirms a risk locus shared with amyotrophic lateral sclerosis. *Neurobiol Aging*. 2011;32(4):758, e1-e7.
16. Renton AE, Majounie E, Waite A, et al; ITALSGEN Consortium. A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. *Neuron*. 2011;72(2):257-268.
17. DeJesus-Hernandez M, Mackenzie IR, Boeve BF, et al. Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. *Neuron*. 2011;72(2):245-256.
18. Gijselinck I, Van Langenhove T, van der Zee J, et al. A C9orf72 promoter repeat expansion in a Flanders-Belgian cohort with disorders of the frontotemporal lobar degeneration-amyotrophic lateral sclerosis spectrum: a gene identification study. *Lancet Neurol*. 2012;11(1):54-65.
19. Al-Sarraj S, King A, Troakes C, et al. p62 Positive, TDP-43 negative, neuronal cytoplasmic and intranuclear inclusions in the cerebellum and hippocampus define the pathology of C9orf72-linked FTLD and MND/ALS. *Acta Neuropathol*. 2011; 122(6):691-702.
20. Hsiung GY, DeJesus-Hernandez M, Feldman HH, et al. Clinical and pathological features of familial frontotemporal dementia caused by C9ORF72 mutation on chromosome 9p. *Brain*. 2012;135(pt 3):709-722.
21. Clinical and neuropathological criteria for frontotemporal dementia: the Lund and Manchester Groups. *J Neurol Neurosurg Psychiatry*. 1994;57(4):416-418.
22. Brooks BR, Miller RG, Swash M, Munsat TL; World Federation of Neurology Research Group on Motor Neuron Diseases. El Escorial revisited: revised criteria for the diagnosis of amyotrophic lateral sclerosis. *Amyotroph Lateral Scler Other Motor Neuron Disord*. 2000;1(5):293-299.
23. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology*. 1984;34(7):939-944.
24. Hughes AJ, Daniel SE, Lees AJ. Improved accuracy of clinical diagnosis of Lewy body Parkinson's disease. *Neurology*. 2001;57(8):1497-1499.
25. Marras C, Schüle B, Munhoz RP, et al. Phenotype in parkinsonian and nonparkinsonian LRRK2 G2019S mutation carriers [published correction appears in *Neurology*. 2011;77(15):1501]. *Neurology*. 2011;77(4):325-333.
26. Bernardi L, Maletta RG, Tomaino C, et al. The effects of APOE and tau gene variability on risk of frontotemporal dementia. *Neurobiol Aging*. 2006;27(5):702-709.
27. Sham PC, Curtis D. Monte Carlo tests for associations between disease and alleles at highly polymorphic loci. *Ann Hum Genet*. 1995;59(pt 1):97-105.
28. McKnight AJ, Maxwell AP, Sawcer S, et al. A genome-wide DNA microsatellite association screen to identify chromosomal regions harboring candidate genes in diabetic nephropathy. *J Am Soc Nephrol*. 2006;17(3):831-836.
29. Simón-Sánchez J, Dopper EG, Cohn-Hokke PE, et al. The clinical and pathological phenotype of C9ORF72 hexanucleotide repeat expansions. *Brain*. 2012; 135(pt 3):723-735.
30. Troakes C, Maekawa S, Wijesekera L, et al. An MND/ALS phenotype associated with C9orf72 repeat expansion: abundant p62-positive, TDP-43-negative inclusions in cerebral cortex, hippocampus and cerebellum but without associated cognitive decline [published online December 19, 2011]. *Neuropathology*. doi: 10.1111/j.1440-1789.2011.01286.x.
31. Snowden JS, Rollinson S, Thompson JC, et al. Distinct clinical and pathological characteristics of frontotemporal dementia associated with C9ORF72 mutations. *Brain*. 2012;135(pt 3):693-708.
32. Boeve BF, Boylan KB, Graff-Radford NR, et al. Characterization of frontotemporal dementia and/or amyotrophic lateral sclerosis associated with the GGGGCC repeat expansion in C9ORF72. *Brain*. 2012;135(pt 3):765-783.
33. Murray ME, DeJesus-Hernandez M, Rutherford NJ, et al. Clinical and neuropathologic heterogeneity of c9FTD/ALS associated with hexanucleotide repeat expansion in C9ORF72. *Acta Neuropathol*. 2011;122(6):673-690.
34. Byrne S, Elamin M, Bede P, et al. Cognitive and clinical characteristics of patients with amyotrophic lateral sclerosis carrying a C9orf72 repeat expansion: a population-based cohort study. *Lancet Neurol*. 2012;11(3):232-240.
35. Mathias JL, Morphett K. Neurobehavioral differences between Alzheimer's disease and frontotemporal dementia: a meta-analysis. *J Clin Exp Neuropsychol*. 2010;32(7):682-698.
36. Spillantini MG, Bird TD, Ghetti B. Frontotemporal dementia and Parkinsonism linked to chromosome 17: a new group of tauopathies. *Brain Pathol*. 1998; 8(2):387-402.
37. Jellinger KA. Neuropathological aspects of Alzheimer disease, Parkinson disease and frontotemporal dementia. *Neurodegener Dis*. 2008;5(3-4):118-121.
38. Jellinger KA. Interaction between pathogenic proteins in neurodegenerative disorders. *J Cell Mol Med*. 2012;16(6):1166-1183.
39. Paisán-Ruíz C, Lang AE, Kawarai T, et al. LRRK2 gene in Parkinson disease: mutation analysis and case control association study. *Neurology*. 2005;65(5): 696-700.
40. Coffey SM, Cook K, Tartaglia N, et al. Expanded clinical phenotype of women with the FMR1 premutation. *Am J Med Genet A*. 2008;146A(8):1009-1016.
41. Bourgeois JA, Coffey SM, Rivera SM, et al. A review of fragile X premutation disorders: expanding the psychiatric perspective. *J Clin Psychiatry*. 2009;70(6): 852-862.
42. Majounie E, Abramzon Y, Renton AE, et al. Repeat expansion in C9ORF72 in Alzheimer's disease. *N Engl J Med*. 2012;366(3):283-284.

ELSEVIER

Neurobiology of Aging 33 (2012) 1851.e17–1851.e19

NEUROBIOLOGY OF AGING

www.elsevier.com/locate/neuaging

Expansion mutation in *C9ORF72* does not influence plasma progranulin levels in frontotemporal dementia

Oriol Dols-Icardo[a,b,1], Marc Suárez-Calvet[a,b,1], Isabel Hernández[c], Guillermo Amer[d], Sofía Antón-Aguirre[a,b], Daniel Alcolea[a,b], Juan Fortea[a,b], Mercè Boada[c], Lluís Tárraga[c], Rafael Blesa[a,b], Alberto Lleó[a,b], Jordi Clarimón[a,b,*]

[a] *Department of Neurology, Hospital de la Santa Creu i Sant Pau, Universitat Autònoma de Barcelona, Barcelona, Spain*
[b] *Center for Networker Biomedical Research in Neurodegenerative Diseases, Madrid, Spain*
[c] *Memory Clinic of Fundació ACE, Institut Català de Neurociències Aplicades, Barcelona, Spain*
[d] *Department of Neurology, Hospital Universitari Son Espases, Mallorca, Spain*

Received 23 January 2012; received in revised form 28 February 2012; accepted 8 March 2012

Abstract

A hexanucleotide repeat expansion in chromosome 9 open reading frame 72 (*C9ORF72*) gene has recently been described as a cause of familial and sporadic frontotemporal lobar degeneration. The aim of this study was to assess whether plasma progranulin (GRN) levels could be modulated by the presence of this repeat expansion. Sixty-five patients diagnosed with frontotemporal dementia and 10 family members with familial aggregation of disease were screened for the presence of the hexanucleotide repeat expansion in *C9ORF72* gene, using a repeat-primed polymerase chain reaction method. Plasma GRN levels were measured in all subjects through enzyme-linked immunosorbent assay. Seven individuals with the repeat expansion were identified. No differences were found between *C9ORF72* repeat expansion carriers and noncarriers (116.4 ± 21 ng/mL and 131.7 ± 36 ng/mL, respectively, $p = 0.3$). Analysis of family members did not disclose any difference in plasma GRN levels between carriers and noncarriers. In conclusion, plasma GRN levels are not influenced by the hexanucleotide repeat expansion in *C9ORF72* gene, and therefore, cannot be used as a reliable biomarker to detect mutation carriers.

Keywords: Frontotemporal dementia; Progranulin; *C9ORF72*; Genetics; Biomarkers

1. Introduction

Frontotemporal lobar degeneration (FTLD) comprises a group of clinically heterogeneous neurodegenerative diseases with diverse neuropathological and genetic substrates. Currently, FTLD is pathologically classified based on the three main proteins that aggregate in the central nervous system: transactive response (TAR) DNA-binding protein43 (TDP-43), microtubule-associated protein tau (MAPT), and fused in sarcoma (Mackenzie et al., 2010).

Mutations in the progranulin gene (*GRN*; MIM: 138945) are the most common genetic cause of FTLD and are typically associated with TDP-43 pathology (Cruts et al., 2006). Most *GRN* mutations cause protein haploinsufficiency, leading to a significant decrease in progranulin (GRN) levels that can be detected in plasma of mutation carriers (Ghidoni et al., 2008). A hexanucleotide (GGGGCC) repeat expansion in the noncoding region of chromosome 9 open reading frame 72 gene (*C9ORF72*; MIM: 614260) was recently found to be a major cause of familial and sporadic FTLD (DeJesus-Hernandez et al., 2011; Renton et al., 2011). All the cases with this mutation described to date show TDP-43 pathology in the central nervous system (Murray et al., 2011). The fact that *GRN* mutations and *C9ORF72* expansions both show TDP-43 pathology suggests they may share some disease mecha-

* Corresponding author at: Department of Neurology, Hospital de la Santa Creu i Sant Pau, Sant Antoni M. Claret 167, Barcelona 08025, Spain. Tel.: +34 932919050; fax: +34 935565602.
E-mail address: jclarimon@santpau.cat (J. Clarimón).
[1] These authors contributed equally to this work.

0197-4580/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
10.1016/j.neurobiolaging.2012.03.005

nisms. It has been described that plasma GRN levels reflect mutation status and that they can be modulated by some additional factors, such as sortilin levels or genetic variation within *TMEM106B* gene (MIM: 613413) (Carrasquillo et al., 2010; Finch et al., 2011). As low levels of GRN might lead to FTLD, investigating modifiers of GRN levels may be important for the development of therapeutic strategies.

This study was aimed to test whether *C9ORF72* expansion mutation could influence GRN levels in patients with frontotemporal dementia (FTD). For this purpose, we included a series of 65 Spanish patients of Caucasian origin followed at two referral centers for dementia in Barcelona ("Sant Pau" Hospital and "Fundació ACE"). All patients were evaluated by two neurologists and those who were testable underwent a thorough neuropsychological assessment. Diagnosis was made using the new international consensus criteria for behavioral variant of frontotemporal dementia (bvFTD) (Rascovsky et al., 2011) and for primary progressive aphasia (Leyton et al., 2011). Thirty patients (46.2%) were women, and the mean age at disease onset was 65.2 ± 10 years. Thirty-seven patients (56.9%) met criteria for bvFTD, 10 (15.4%) for semantic dementia, and 18 (27.7%) for progressive nonfluent aphasia. We also included three FTD patients and seven unaffected first-degree family members from a Spanish family from the Balearic Islands (referred from the Son Espases Hospital, Mallorca). All patients or their relatives gave informed consent. This research was approved by the ethics committee at all three centers. Mutations in *MAPT* (MIM: 157140) and *GRN* genes were analyzed in all patients with at least one affected first-degree family member (n = 22; 33.8%) by direct sequencing of the coding region and exon–intron boundaries for exons 2 and 9–13 of *MAPT* gene and for the 12 coding exons of *GRN* gene. A repeat-primed polymerase chain reaction method was used to screen all patients for the presence of the GGGGCC hexanucleotide repeat expansion in *C9ORF72* gene, following a previously described method with minor modifications (Renton et al., 2011), and a threshold of 30 repeats was used as a cutoff to discriminate between expansion carriers and noncarriers (DeJesus-Hernandez et al., 2011; Renton et al., 2011). Plasma GRN levels were measured in duplicate in all patients using the Human Progranulin ELISA Kit (Adipogen, Inc., Seoul, Korea) following the manufacturer's instructions. "Interassay" coefficient of variation was 8.4%. Plasma GRN levels were compared between expansion carriers and noncarriers using a Mann–Whitney test. Data were analyzed with Statistical Package for Social Science v19.0 (SPSS, Inc., Chicago, IL).

A hexanucleotide repeat expansion in *C9ORF72* gene was found in seven individuals. Three of them belonged to the 65 patients included in the FTD series and all had positive family history of dementia, thus representing 13.6% of familial cases. The repeat expansion was also found in 4 of the 10 Balearic Islands family members, one being a 25-year-old asymptomatic individual. All patients carrying the expansion mutation presented with bvFTD. Additionally, one patient with semantic dementia carried a *GRN* mutation (c.709–1 G>A), which has been previously described as a pathogenic mutation resulting in GRN haploinsufficiency (López de Munain et al., 2008). Patients not carrying the expansion had a maximum of 14 hexanucleotide repeats.

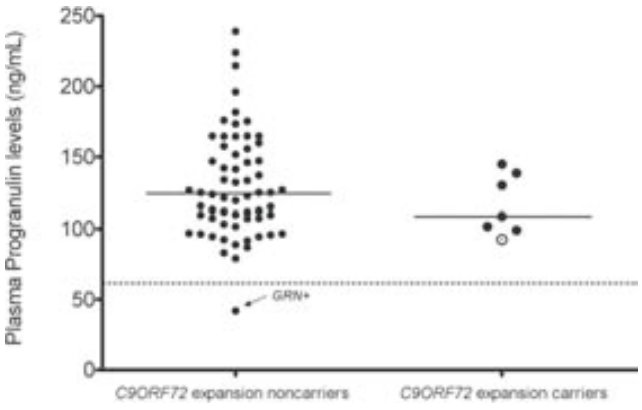


Fig. 1. Plasma progranulin (GRN) levels (ng/mL) of carriers (n = 7) and noncarriers (n = 62) of the hexanucleotide repeat expansion in *C9ORF72* gene. The horizontal line represents the median plasma GRN levels in each group, the white circle designates the asymptomatic expansion carrier, the arrow indicates the patient carrying the *GRN* mutation (c.709-1 G>A), and the dashed line represents the cutoff threshold (61.55 ng/mL), which has been reported to best correlate with GRN haploinsufficiency (Ghidoni et al., 2012).

Analysis of plasma GRN levels in all FTD patients (excluding the *GRN* mutation carrier) disclosed an overall average of 130.1 ± 35 ng/mL. GRN levels were not influenced by age at blood draw or gender. Carriers of the hexanucleotide repeat expansion (n = 7) had average plasma GRN levels of 116.4 ± 21 ng/mL. These levels did not differ from those in patients not carrying the expansion mutation (131.7 ± 36 ng/mL, $p = 0.3$, n = 61) (Fig. 1). No differences in plasma GRN levels were found between the four carriers and the six noncarriers of the Balearic Islands family either ($p = 0.5$). Only the patient carrying the GRN c.709–1 G>A mutation showed plasma GRN levels lower than 61.55 ng/mL, the level proposed as the cutoff threshold that best correlates with GRN haploinsufficiency (Ghidoni et al., 2012).

In summary, our results support that *C9ORF72* hexanucleotide expansion does not influence plasma GRN levels and suggest that plasma GRN levels do not appear to be a reliable biomarker for the detection of FTD associated to *C9ORF72* repeat expansion.

Disclosure statement

The authors declare that there are no actual or potential conflicts of interest.

Acknowledgements

The authors are indebted to all the patients and their families for their participation. They thank Dr. Agustín Ruiz for his helpful remarks, Carolyn Newey for editorial help, and Laia Muñoz and Inés M. Matas for technical assistance. This work was supported by a research grant from "Center for Networker Biomedical Research in Neurodegenerative Diseases" and by the "Fondo de Investigaciones Sanitarias" (PI09/00098).

References

Carrasquillo, M.M., Nicholson, A.M., Finch, N., Gibbs, J.R., Baker, M., Rutherford, N.J., Hunter, T.A., DeJesus-Hernandez, M., Bisceglio, G.D., Mackenzie, I.R., Singleton, A., Cookson, M.R., Crook, J.E., Dillman, A., Hernandez, D., Petersen, R.C., Graff-Radford, N.R., Younkin, S.G., Rademakers, R., 2010. Genome-wide screen identifies rs646776 near sortilin as a regulator of progranulin levels in human plasma. Am. J. Hum. Genet. 87, 890–897.

Cruts, M., Gijselinck, I., van der Zee, J., Engelborghs, S., Wils, H., Pirici, D., Rademakers, R., Vandenberghe, R., Dermaut, B., Martin, J.J., van Duijn, C., Peeters, K., Sciot, R., Santens, P., De Pooter, T., Mattheijssens, M., Van den Broeck, M., Cuijt, I., Vennekens, K., De Deyn, P.P., Kumar-Singh, S., Van Broeckhoven, C., 2006. Null mutations in progranulin cause ubiquitin-positive frontotemporal dementia linked to chromosome 17q21. Nature 442, 920–924.

DeJesus-Hernandez, M., Mackenzie, I.R., Boeve, B.F., Boxer, A.L., Baker, M., Rutherford, N.J., Nicholson, A.M., Finch, N.A., Flynn, H., Adamson, J., Kouri, N., Wojtas, A., Sengdy, P., Hsiung, G.Y., Karydas, A., Seeley, W.W., Josephs, K.A., Coppola, G., Geschwind, D.H., Wszolek, Z.K., Feldman, H., Knopman, D.S., Petersen, R.C., Miller, B.L., Dickson, D.W., Boylan, K.B., Graff-Radford, N.R., Rademakers, R., 2011. Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. Neuron 72, 245–256.

Finch, N., Carrasquillo, M.M., Baker, M., Rutherford, N.J., Coppola, G., Dejesus-Hernandez, M., Crook, R., Hunter, T., Ghidoni, R., Benussi, L., Crook, J., Finger, E., Hantanpaa, K.J., Karydas, A.M., Sengdy, P., Gonzalez, J., Seeley, W.W., Johnson, N., Beach, T.G., Mesulam, M., Forloni, G., Kertesz, A., Knopman, D.S., Uitti, R., White, C.L. 3rd, Caselli, R., Lippa, C., Bigio, E.H., Wszolek, Z.K., Binetti, G., Mackenzie, I.R., Miller, B.L., Boeve, B.F., Younkin, S.G., Dickson, D.W., Petersen, R.C., Graff-Radford, N.R., Geschwind, D.H., Rademakers, R., 2011. TMEM106B regulates progranulin levels and the penetrance of FTLD in GRN mutation carriers. Neurology 76, 467–474.

Ghidoni, R., Benussi, L., Glionna, M., Franzoni, M., Binetti, G., 2008. Low plasma progranulin levels predict progranulin mutations in frontotemporal lobar degeneration. Neurology 71, 1235–1239.

Ghidoni, R., Stoppani, E., Rossi, G., Piccoli, E., Albertini, V., Paterlini, A., Glionna, M., Pegoiani, E., Agnati, L.F., Fenoglio, C., Scarpini, E., Galimberti, D., Morbin, M., Tagliavini, F., Binetti, G., Benussi, L., 2012. Optimal Plasma Progranulin Cutoff Value for Predicting Null Progranulin Mutations in Neurodegenerative Diseases: A Multicenter Italian Study. Neurodegener. Dis. 9, 121–127.

Leyton, C.E., Villemagne, V.L., Savage, S., Pike, K.E., Ballard, K.J., Piguet, O., Burrell, J.R., Rowe, C.C., Hodges, J.R., 2011. Subtypes of progressive aphasia: application of the International Consensus Criteria and validation using beta-amyloid imaging. Brain 134, 3030–3043.

López de Munain, A., Alzualde, A., Gorostidi, A., Otaegui, D., Ruiz-Martínez, J., Indakoetxea, B., Ferrer, I., Pérez-Tur, J., Sáenz, A., Bergareche, A., Barandiarán, M., Poza, J.J., Zabalza, R., Ruiz, I., Urtasun, M., Fernández-Manchola, I., Olasagasti, B., Espinal, J.B., Olaskoaga, J., Ruibal, M., Moreno, F., Carrera, N., Martí-Massó, J.F., 2008. Mutations in progranulin gene: clinical, pathological, and ribonucleic acid expression findings. Biol. Psychiatry 63, 946–952.

Mackenzie, I.R., Neumann, M., Bigio, E.H., Cairns, N.J., Alafuzoff, I., Kril, J., Kovacs, G.G., Ghetti, B., Halliday, G., Holm, I.E., Ince, P.G., Kamphorst, W., Revesz, T., Rozemuller, A.J., Kumar-Singh, S., Akiyama, H., Baborie, A., Spina, S., Dickson, D.W., Trojanowski, J.Q., Mann, D.M., 2010. Nomenclature and nosology for neuropathologic subtypes of frontotemporal lobar degeneration: an update. Acta. Neuropathol. 119, 1–4.

Murray, M.E., DeJesus-Hernandez, M., Rutherford, N.J., Baker, M., Duara, R., Graff-Radford, N.R., Wszolek, Z.K., Ferman, T.J., Josephs, K.A., Boylan, K.B., Rademakers, R., Dickson, D.W., 2011. Clinical and neuropathologic heterogeneity of c9FTD/ALS associated with hexanucleotide repeat expansion in C9ORF72. Acta. Neuropathol. 122, 673–690.

Rascovsky, K., Hodges, J.R., Knopman, D., Mendez, M.F., Kramer, J.H., Neuhaus, J., van Swieten, J.C., Seelaar, H., Dopper, E.G., Onyike, C.U., Hillis, A.E., Josephs, K.A., Boeve, B.F., Kertesz, A., Seeley, W.W., Rankin, K.P., Johnson, J.K., Gorno-Tempini, M.L., Rosen, H., Prioleau-Latham, C.E., Lee, A., Kipps, C.M., Lillo, P., Piguet, O., Rohrer, J.D., Rossor, M.N., Warren, J.D., Fox, N.C., Galasko, D., Salmon, D.P., Black, S.E., Mesulam, M., Weintraub, S., Dickerson, B.C., Diehl-Schmid, J., Pasquier, F., Deramecourt, V., Lebert, F., Pijnenburg, Y., Chow, T.W., Manes, F., Grafman, J., Cappa, S.F., Freedman, M., Grossman, M., Miller, B.L., 2011. Sensitivity of revised diagnostic criteria for the behavioural variant of frontotemporal dementia. Brain 134, 2456–2477.

Renton, A.E., Majounie, E., Waite, A., Simón-Sánchez, J., Rollinson, S., Gibbs, J.R., Schymick, J.C., Laaksovirta, H., van Swieten, J.C., Myllykangas, L., Kalimo, H., Paetau, A., Abramzon, Y., Remes, A.M., Kaganovich, A., Scholz, S.W., Duckworth, J., Ding, J., Harmer, D.W., Hernandez, D.G., Johnson, J.O., Mok, K., Ryten, M., Trabzuni, D., Guerreiro, R.J., Orrell, R.W., Neal, J., Murray, A., Pearson, J., Jansen, I.E., Sondervan, D., Seelaar, H., Blake, D., Young, K., Halliwell, N., Callister, J.B., Toulson, G., Richardson, A., Gerhard, A., Snowden, J., Mann, D., Neary, D., Nalls, M.A., Peuralinna, T., Jansson, L., Isoviita, V.M., Kaivorinne, A.L., Hölttä-Vuori, M., Ikonen, E., Sulkava, R., Benatar, M., Wuu, J., Chiò, A., Restagno, G., Borghero, G., Sabatelli, M., ITALSGEN Consortium, Heckerman, D., Rogaeva, E., Zinman, L., Rothstein, J.D., Sendtner, M., Drepper, C., Eichler, E.E., Alkan, C., Abdullaev, Z., Pack, S.D., Dutra, A., Pak, E., Hardy, J., Singleton, A., Williams, N.M., Heutink, P., Pickering-Brown, S., Morris, H.R., Tienari, P.J., Traynor, B.J., 2011. A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. Neuron 72, 257–268.

JNNP Online First, published on December 4, 2013 as 10.1136/jnnp-2013-305972

RESEARCH PAPER

Plasma phosphorylated TDP-43 levels are elevated in patients with frontotemporal dementia carrying a *C9orf72* repeat expansion or a *GRN* mutation

Marc Suárez-Calvet,[1,2] Oriol Dols-Icardo,[1,2] Albert Lladó,[3] Raquel Sánchez-Valle,[3] Isabel Hernández,[4] Guillermo Amer,[5] Sofía Antón-Aguirre,[1,2] Daniel Alcolea,[1,2] Juan Fortea,[1,2] Isidre Ferrer,[2,6] Julie van der Zee,[7,8] Lubina Dillen,[7,8] Christine Van Broeckhoven,[7,8] José Luís Molinuevo,[3] Rafael Blesa,[1,2] Jordi Clarimón,[1,2] Alberto Lleó[1,2]

▶ Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/jnnp-2013-305972).

For numbered affiliations see end of article.

Correspondence to
Dr Alberto Lleó, Neurology Department, Hospital de la Santa Creu i Sant Pau, Sant Antoni M Claret 167, Barcelona 08025, Spain; alleo@santpau.es

Received 28 May 2013
Revised 27 September 2013
Accepted 29 October 2013

ABSTRACT

Objectives About a half of patients with frontotemporal dementia (FTD) has deposition of phosphorylated TDP-43 protein (pTDP-43) in the brain. We studied pTDP-43 and total TDP-43 levels in plasma and cerebrospinal fluid (CSF) in healthy controls and patients with FTD, including those carrying a repeat expansion in the *C9orf72* gene or a mutation in *GRN*.

Methods We included 88 plasma samples of 10 *C9orf72* expansion carriers, 5 *GRN* mutation carriers, 51 patients with FTD without a known mutation and 22 healthy controls. We also obtained CSF samples from 25 patients with FTD (2 with *C9orf72* expansion and 3 with a *GRN* mutation) and 22 healthy controls. We measured pTDP-43 and total TDP-43 levels using sandwich ELISA.

Results Patients carrying the *C9orf72* repeat expansion or a *GRN* mutation had significantly higher plasma and CSF levels of pTDP-43 than the remaining patients with FTD ($p<0.05$). In addition, plasma pTDP-43 levels were higher in patients with FTD carrying a *C9orf72* expansion or *GRN* mutations than in healthy controls ($p<0.05$).

Conclusions Our study shows that plasma pTDP-43 levels may be increased in some genetic forms of FTD known to be associated with TDP-43 proteinopathies.

INTRODUCTION

Frontotemporal dementia (FTD) comprises a group of clinical syndromes characterised by varying degrees of behavioural disturbances, personality changes and language impairment.[1] Based on the topographical distribution and networks involved, three syndromes have been described: behavioural variant FTD (bvFTD), semantic dementia (SD) and progressive non-fluent aphasia (PNFA).[2] These clinical syndromes overlap with some extrapyramidal syndromes and motor neuron disease (MND). FTD syndromes are associated with a spectrum of underlying histopathologies usually grouped under the umbrella term of frontotemporal lobar degeneration (FTLD).[3] The neuropathology of FTLD is heterogeneous and most cases can be classified according to the protein deposited in inclusion bodies in the central nervous system.[4] According to this nomenclature, cases can be assigned to one of three major molecular subgroups: FTLD-TDP, FTLD-tau or FTLD-fused in sarcoma (FUS), depending on whether inclusions contain TAR DNA-binding protein-43 (TDP-43), tau, or FUS, respectively. Family history is common in FTD, suggesting a strong genetic influence.[5] Mutations in *GRN* (MIM: 138945) and *MAPT* (MIM: 157140) genes have been described to cause FTD.[3 6–8] Recently, a hexanucleotide repeat expansion in *C9orf72* (MIM: 614260) has been shown to be the most common genetic cause of FTD and MND.[9–12]

TDP-43 was identified in 2006 to be one of the major protein components of the ubiquitinated inclusions present in approximately half of the cases of FTLD and most cases of amyotrophic lateral sclerosis (ALS).[13 14] Mutations in the *TARDBP* (MIM: 605078) gene, which encodes for TDP-43, were subsequently identified in a few cases of ALS and FTD-MND.[15 16] In spite of their low frequency, mutations in *TARDBP* confirm the primary role of the protein in disease pathogenesis. The presence of TDP-43 inclusions as a protein signature across several neurodegenerative disorders has led to the adoption of the term TDP-43 proteinopathies. Despite the varying clinical presentation of each TDP-43 proteinopathy, they possibly share a common disease mechanism, biomarkers and therapeutic strategies. It is still difficult, however, to accurately predict the presence of a TDP-43 proteinopathy based on the clinical syndrome alone. Therefore, the search for biomarkers for TDP-43 proteinopathies would greatly facilitate early diagnosis and help in the differential diagnosis of FTLD-TDP with other forms of FTLD or other neurodegenerative diseases, a critical step for the introduction of disease-modifying drugs.[17] Previous studies have investigated the role of plasma and cerebrospinal fluid (CSF) TDP-43 levels as a biomarker in patients with FTD or ALS, with variable results.[18–21] In one of these studies,[19] an ELISA was also developed to measure TDP-43 protein phosphorylated at the residues S409/410, which appeared to be more reliable than total TDP-43 to distinguish FTLD-TDP from other forms of FTLD or from Alzheimer's disease (AD). However, whether phosphorylated TDP-43 (pTDP-43) is increased in CSF or in plasma of genetic forms of FTD remains unknown.

In the present study, we measured pTDP-43 and total TDP-43 levels in plasma and in CSF in a

To cite: Suárez-Calvet M, Dols-Icardo O, Lladó A, *et al*. *J Neurol Neurosurg Psychiatry* Published Online First: [*please include* Day Month Year] doi:10.1136/jnnp-2013-305972

group of patients with FTD and in controls. We included subjects with mutations in *GRN* and subjects with the hexanucleotide repeat expansion in the *C9orf72* gene, both disorders known to be TDP-43 proteinopathies.

PATIENTS AND METHODS

Patients

We included patients diagnosed with FTD followed in four specialised neurological referral centres in Spain (HSCSP, HC, FACE, HSE). Some of the samples were obtained from patients enrolled in the Genetic Counselling Program for familial dementias at Hospital Clínic, Barcelona.[22] All patients diagnosed with bvFTD fulfilled the new revised criteria[23] and those with SD or PNFA accomplished the primary progressive aphasia international consensus criteria.[24] All patients and their caregivers were evaluated by a neurologist with experience in neurodegenerative diseases, who reviewed the medical and neurological history, and performed a complete neurological examination. Healthy control subjects were selected among the patients' caregivers. The control subjects had no known neurological or psychiatric illness or memory or other cognitive complaints, and they showed normal performance for age in neuropsychological testing. Plasma pTDP-43 levels were determined in 88 subjects: 10 carriers of the *C9orf72* repeat expansion (7 bvFTD, 2 FTD-MND and 1 asymptomatic individual), 5 symptomatic *GRN* mutation carriers (two cases with *GRN* p.C366fsX1, one with *GRN* p.V279GfsX5, one with *GRN* c.709-1G>A and another with *GRN* p.C139R), 51 patients with a clinical diagnosis of FTD and 22 healthy controls (table 1). Among the 51 patients with FTD, 27 (52.9%) had bvFTD (two with bvFTD-MND), 7 (13.7%) had SD and 17 (33.3%) had PNFA. In this group, a *C9orf72* repeat expansion had been excluded and mutations in *GRN* and *MAPT* had been discarded in those with family history of dementia. Among the five patients with a *GRN* mutation, three fulfilled criteria for bvFTD, one for SD and the other for PNFA. Some of these cases have been published previously.[25–27]

CSF samples from 25 patients with a clinical diagnosis of FTD and 22 control subjects were also included. Two patients with FTD carried a *C9orf72* repeat expansion and three subjects carried a *GRN* mutation (two cases with the *GRN* p.C366fsX1 and one with the *GRN* p.C139R mutation, table 2).

Plasma and/or CSF samples were obtained after written informed consent was given by the subjects or their relatives. The local ethical standards committee on human experimentation approved the study.

Plasma and CSF collection and processing

Blood samples were drawn and processed as previously described.[26] CSF was obtained by lumbar puncture following standard procedures, collected in a 15 mL polypropylene tube, and processed in less than 30 min. Samples were centrifuged at 1700 g for 10 min (4°C) and immediately frozen at −80°C until use. All CSF samples were analysed for total tau (t-tau), phospho tau (p-tau), and amyloid-β_{42} ($A\beta_{42}$) by ELISA (INNOTEST, Innogenetics, Ghent, Belgium) following the manufacturer's instructions. None of the patients or control subjects included had an AD CSF profile defined by Mattsson *et al*[28] equation ($A\beta_{42}$/p-tau)/(3.694+0.0105x t-tau).

Phosphorylated TDP-43 ELISA assay

Plasma and CSF pTDP-43 levels were measured in duplicate using a commercially available ELISA kit (pTDP-43 ELISA Kit, E9442h EIAab, Wuhan, China) following the manufacturer's instructions. This assay uses a rabbit polyclonal antibody raised against human TDP-43 protein as a capture antibody and a biotinylated rabbit polyclonal antibody against TDP-43 phosphorylated at Ser409 (antiphospho-TDP-43 (pSer409), Cat. Num. SAB4200223 Sigma-Aldrich, Saint Louis, Missouri, USA) as a detection antibody. The standard provided by the manufacturer consists of recombinant human TDP-43 phosphorylated at Ser409. Due to the absence of a well-established assay for TDP-43, we expressed the results as relative units generated from concentration values normalised to a control sample (plasma or CSF) loaded onto all plates. The interassay coefficients of variation for plasma and CSF measurements were 25.4% and 17.1%, respectively. The plasma and CSF samples were tested undiluted. We performed a *4-parameter logistic* fit

Table 1 Characteristics of the study patients with plasma measurements

Variable	CONTROL (n=22)	FTD (n=51)*	*C9orf72* (n=10)	*GRN* (n=5)	p Value
Gender M/F—no. (%)	9 (41)/13 (59)	27 (53)/24 (47)	7 (70)/3 (30)	1 (20)/4 (80)	0.230
Age—year (mean±SD)	70.1±9.6	67.8±8.8	52.4±9.2	65.2±6.1	<0.001†
Age of onset of the disease—year (mean±SD)	NA	62.9±9.4	48.8±8.8	61.2±4.3	<0.001†
Disease duration year—(mean±SD)	NA	5.0±3.5	3.7±2.4	4.00±2.7	0.490
MMSE– Median (IQR)	29 (28.75–30)	11 (3–24)	26 (22–27.25)	15 (9.5–27.25)	<0.001†
GDS– Median (IQR)	1 (1)	5 (4–6)	4.5 (3–5)	4 (4–5)	<0.001†
Plasma pTDP-43 levels (relative units)					
Median	0.052	0.120	1.574‡	2.970‡	0.002†
(IQR)	(0.000–0.279)	(0.020–0.829)	(0.329–9.732)	(1.014–8.706)	
Total TDP-43 levels (relative units)					
Median	4.370	5.896	1.488§	0.755§	0.008†
(IQR)	(3.190–6.925)	(3.588–9.396)	(1.012–7.225)	(0.454–5.254)	

Data are expressed as mean±SD, number of patients (per cent) or median (IQR), as appropriate. Plasma pTDP-43 and total TDP-43 levels are expressed as values relative to an internal control sample. Probability values (p) denote differences between control, FTD, *C9orf72* and *GRN* patients groups, except for 'age of onset of the disease' and 'disease duration' where control group is excluded of the analysis. In these variables, the asymptomatic patient in the *C9orf72* was not included. χ^2 statistics were used for categorical variables. One-way ANOVA was used to compare continuous variables between groups. MMSE, GDS, pTDP-43 and total TDP-43 were evaluated by non-parametric statistical analysis (Kruskal-Wallis).
*Total TDP-43 levels were measured in 50 patients with FTD.
†Significant difference (p<0.05).
‡p<0.05 in post hoc comparison between *C9orf72* or *GRN* with control and FTD groups (Mann-Whitney).
§p<0.05 in post hoc comparison between *GRN* and control or FTD groups and *C9orf72* and FTD group (Mann-Whitney).
ANOVA, analysis of variance; FTD, frontotemporal dementia; GDS, Global Deterioration Scale; MMSE, Mini-Mental State Examination; IQR, interquartile range; NA, not applicable.

Table 2 Baseline characteristics of the study patients with CSF measurements.

Variable	CONTROL (n=22)*	FTD (n=20)	*C9orf72* (n=2)	*GRN* (n=3)	p Value
Gender M/F—no. (%)	13 (59)/9 (41)	13 (65)/7 (35)	2 (100)/0 (0)	1 (33)/2 (67)	0.490
Age—year (mean±SD)	64.82±8.3	63.65±10.1	57.50±9.2	62.00±4.6	0.710
Age of onset of the disease—year (mean±SD)	NA	59.90±10.7	55.00±7.1	58.33±2.1	0.794
Disease duration year—(mean±SD)	NA	3.73±2.8	2.50±2.1	3.67±2.9	0.842
MMSE– Median (IQR)	29 (28–29)	22.5 (15.5–26.75)	26–27	25(15–30)	<0.001†
GDS– Median (IQR)	1 (1–1)	4 (4–5)	3–4	4(4–5)	<0.001†
CSF biomarkers					
Aβ 1–42—pg/mL (mean±SD)	639±248	548±204	577.9±14	640±193	0.609
P-tau—pg/mL (mean±SD)	48.8±13	38.8±16	48.5±3	38.2±3	0.122
T-tau—pg/mL (mean±SD)	238±80	225±94	228±74	231±40	0.968
CSF pTDP-43 (relative units)					
Median	1.267	1.183	1.781	1.749	0.028†
(IQR)	(1.12–1.60)	(1.01–1–40)	NA	NA	
CSF total TDP-43 (relative units)					
Median	0.569	0.290	0.601	0.825	0.466
(IQR)	(0.067–0.960)	(0.000–0.861)	NA	NA	

Data are expressed as mean±SD, number of patients (per cent) or median (IQR), as appropriate. CSF pTDP-43 and total TDP-43 levels are expressed as values relative to an internal control sample. Probability values (p) denote differences between control, FTD, *C9orf72* and *GRN* patient groups, except for 'age of onset of the disease' and 'disease duration' where control group is excluded of the analysis. χ^2 statistics were used for categorical variables. One-way ANOVA was used to compare continuous variables between groups. MMSE, GDS, pTDP-43 and total TDP-43 levels were evaluated by non-parametric statistical analysis (Kruskal-Wallis).
*Total TDP-43 levels were measured in 16 control subjects.
†Significant difference (p<0.05).
NA, not applicable.
ANOVA, analysis of variance; CSF, cerebrospinal fluid; FTD, frontotemporal dementia; GDS, Global Deterioration Scale; MMSE, Mini-Mental State Examination; IQR, interquartile range; NA, not applicable.

to generate the standard curve, following the manufacturer's recommendations (see online supplementary figure S1). MasterPlex ReaderFit software (MiraiBio Group, Hitachi Solutions America, San Francisco, USA) was used for ELISA calculations.

In order to characterise this ELISA assay, we measured pTDP-43 levels in brain homogenates from two cases with confirmed FTLD-TDP and two controls without any evidence of TDP-43 pathology (see online supplementary figure S2A–F). Brain samples were processed, and soluble and insoluble fractions were obtained. We observed a marked increase in pTDP-43 levels in the insoluble fraction in brain homogenates from FTLD-TDP compared with controls, whereas no difference was found in the soluble fraction (see online supplementary figure S2G).

Total TDP-43 ELISA assay

Total TDP-43 was measured in plasma and CSF using a commercially available ELISA kit (Human TDP-43, KE00005, Proteintech Group, Chicago, USA) following the manufacturer's instructions. The assay uses a rabbit polyclonal antibody raised against amino acids 1-262 of human TDP-43 protein (CatNo. 10782-2-AP, Proteintech Group, Chicago, USA) as a capture antibody and a mouse monoclonal antibody raised against amino acids 203-212 of human TDP-43 (CatNo. 60019-2-Ig, Proteintech Group, Chicago, USA) as a detector antibody. The standard provided by the manufacturer consists of a His-tag recombinant human full-length TDP-43 protein (CatNo. ag13119, Proteintech Group, Chicago, USA). Due to the absence of a well-established assay for TDP-43, we expressed the results as relative units generated from concentration values normalised to a control sample (plasma or CSF) loaded onto all plates. The interassay coefficients of variation for plasma and CSF measurements were 14.3% and 15.8%, respectively. Total TDP-43 levels in human brain homogenates were also measured using this assay. We did not find differences in total TDP-43 levels in either soluble or insoluble fractions between FTLD-TDP cases and controls (see online supplementary figure S2H).

Human brain samples and immunochemistry procedures

Human brain samples were obtained from the 'Institut de Neuropatologia', Hospital Universitari de Bellvitge, Barcelona. We included brain samples from two patients with neuropathological criteria for FTLD-TDP[4] and two age-matched controls (see online supplementary figure S2).

For immunochemistry purposes, dewaxed sections were boiled in citrate buffer (20 min) to retrieve antigenicity. Endogenous peroxidases were blocked by incubation in 10% methanol-1% H_2O_2 solution (15 min) followed by 3% normal horse serum solution. Primary antibodies against TDP-43 (Abnova) and TDP-43 phospho 403-404 (TDP-43P; Cosmo Bio) were used at dilutions of 1:600 and 1:2500, respectively, at 4°C overnight. Following incubation with the primary antibody, the sections were incubated with EnVision+ system peroxidase (Dako Corporation, Glostrup, Denmark) for 30 min at room temperature. The peroxidase reaction was visualised with diaminobenzidine and H_2O_2. The immunoreaction results in a brown precipitate. Sections were slightly counterstained with haematoxylin, dehydrated and cover-slipped for microscopic observation.

For pTDP-43 and total TDP-43 ELISA assays, frozen samples of the frontal cortex from two patients with FTLD-TDP and two controls were used. Tissue was processed using previously described procedures[29] with some modifications. In brief, 100 mg of frozen tissue was homogenised with a mechanical homogeniser in 1 mL of Tris-Triton extraction buffer (Tris 50 mmol/L, pH 7.4, 400 mmol/L NaCl, 2 mmol/L EDTA, 0.1% Triton X-100) with protease inhibitors (Complete; Roche, Indianapolis, Indiana, USA). The soluble fraction was obtained

after centrifugation at 14 000 rpm for 5 min at 4°C. The resulting pellet was resuspended in 70% formic acid and then recentrifuged at 22 000 rpm for 5 min at 4°C. The supernatant was then neutralised with 1 mol/L Tris buffer (pH 11), and used for the ELISA assays. Protein concentration in these samples was determined using the Bradford assay. Soluble fractions were diluted at 1:1000 in the pTDP-43 ELISA assay and at 1:500 in the total TDP-43 ELISA assay. Insoluble fractions were diluted at 1:100 in both assays.

DNA extraction, genetic analysis and Southern blotting

DNA was extracted from whole blood using previously described standard protocols. All patients had been previously investigated for the presence of the GGGGCC hexanucleotide repeat expansion in *C9orf72* gene, and those with a positive family history of dementia were screened for mutations in *GRN* and *MAPT* genes, as previously described.[26]

A non-radioactive Southern blotting protocol was used to estimate the hexanucleotide repeat expansion size.[30] Briefly, a total amount of 20 μg of blood-derived DNA was digested with XbaI and electrophoresed on a 0.8% agarose gel. DNA was transferred to a positively charged nylon membrane (Roche Applied Science) by capillary blotting. Hybridisation was carried out at 45°C overnight using a 954 base pairs probe labelled with digoxigenin. Ready-to-use CDP-*Star* (Roche Applied Science) was used as the chemiluminescent substrate through the transparency technique and signals were visualised on a KODAK Image Station 4000MM PRO (Carestream Health, Rochester, New York, USA). The median number of repeats was used for statistical analysis.

Statistical analysis

The χ^2 test was used to compare differences in categorical variables and one-way analysis of variance (ANOVA) was used to compare age and age at onset between groups. Mini-Mental State Examination (MMSE), Global Deterioration Scale (GDS), pTDP-43 and total TDP-43 levels were evaluated by non-parametric statistical analysis (Kruskal-Wallis and Mann-Whitney post hoc test). Correlation analysis of plasma and CSF pTDP-43 and total TDP-43 levels, age, age of onset, disease duration, MMSE, GDS and median *C9orf72* GGGGCC hexanucleotide repeat number were performed using the Spearman's r test. Statistical significance for all the analyses was set at 5% (α=0.05). All data were analysed using Statistical Package for the Social Sciences (SPSS, Chicago, USA).

RESULTS

Plasma pTDP-43 and total TDP-43 levels in patients with FTD and controls

Table 1 summarises demographic and clinical data of all patients with plasma sample included in this study.

Family history of dementia was present in 39.2% of patients in the FTD group, and in 100% of subjects with the *C9orf72* expansion and *GRN* mutations. Patients carrying the *C9orf72* expansion were significantly younger than the other patients (p<0.001, one-way ANOVA). Their age of onset of the disease was also earlier than that of patients with FTD without mutations (p<0.001, one-way ANOVA). The three groups (*C9orf72*, *GRN* and FTD) did not differ in disease duration or GDS score, but patients with FTD had a lower MMSE, suggesting a more advanced stage of the disease (table 1).

We next investigated whether pTDP-43 plasma levels were influenced by demographic variables. In the entire cohort (n=88), plasma pTDP-43 levels were not influenced by gender (p=0.983, Mann-Whitney) but they correlated inversely with age (Spearman's r=−0.318, p=0.003). In symptomatic subjects (we excluded healthy controls and the *C9orf72* asymptomatic patient, n=65), plasma pTDP-43 levels were not influenced by disease duration (p=0.500, Spearman), MMSE (p=0.070, Spearman) or GDS (p=0.242, Spearman) scores, but they correlated inversely with age of disease onset (Spearman's r=−0.37, p=0.002). No differences in plasma pTDP-43 levels were observed between the three syndromes of FTD (bvFTD, SD, PNFA). When we analysed plasma pTDP-43 levels according to genetic status, we found that levels differed between groups, although there was considerable variability and overlap (p=0.002, Kruskal-Wallis). Subjects carrying a *C9orf72* repeat expansion (n=10) or *GRN* mutations (n=5) had significantly increased levels of plasma pTDP-43 compared with subjects with FTD without a mutation (n=51) and with controls (n=22) (p<0.05, Mann-Whitney, figure 1A, table 1). An ANOVA adjusted for age and age of onset confirmed the statistically significant difference in pTDP-43 levels between patients with the mutation and those without the mutation. We also calculated the upper 99% CI of the control group and found that 6 out of 10 (60%) subjects in the *C9orf72* group and 4 out of 5 (80%) subjects in the *GRN* group were above this value compared with 8 out of 51 (15.7%) in the FTD group (figure 1A). The levels of pTDP-43 in the *C9orf72* and the *GRN* groups did not correlate with age, age of onset, disease duration, FTD subtype or MMSE score. Noticeably, the only asymptomatic subject carrying the *C9orf72* expansion had the lowest plasma pTDP-43 levels (figure 1A).

Next, we measured total TDP-43 levels in plasma in this cohort (except 1 patient with FTD for whom no sample was available; n=87). In the entire group, plasma total TDP-43 levels were not influenced by gender (p=0.610, Mann-Whitney) but they showed a trend towards a positive correlation with age (Spearman's r=+0.21, p=0.051). In symptomatic subjects (n=64), plasma total TDP-43 levels were not influenced by disease duration (p=0.119, Spearman), MMSE (p=0.391, Spearman) or GDS (p=0.966, Spearman) scores, but they correlated positively with age of disease onset (Spearman's r=+0.26, p=0.037). No differences in plasma total TDP-43 levels were observed between the three syndromes of FTD (bvFTD, SD, PNFA). When we analysed plasma total TDP-43 levels according to genetic status, we found that, unlike pTDP-43, total TDP-43 levels were slightly decreased in the *C9orf72* (n=10) and the *GRN* groups (n=5) compared with the FTD group (n=50, p=0.016 and p=0.015, respectively, Mann-Whitney, figure 1B, table 1). Subjects with *GRN* mutations also showed decreased levels of plasma total TDP-43 levels compared with controls (p=0.046, Mann-Whitney). Plasma pTDP-43 levels correlated inversely with plasma total TDP-43 levels in the entire group (Spearman's r=−0.23, p=0.030).

We next tested whether plasma pTDP-43 or total TDP-43 levels were associated with the number of GGGGCC repeats in subjects with the *C9orf72* expansion. To address this issue, we applied a non-radioactive Southern blot technique in blood-derived DNA samples from the 10 subjects carrying the *C9orf72* expansion.[30] We did not find any correlation between plasma pTDP-43 or total TDP-43 levels and the median number of repeats (see online supplementary figure S3).

CSF pTDP-43 and total TDP-43 levels in patients with FTD and controls

We subsequently investigated the differences in pTDP-43 and total TDP-43 levels in CSF. Table 2 shows demographic and

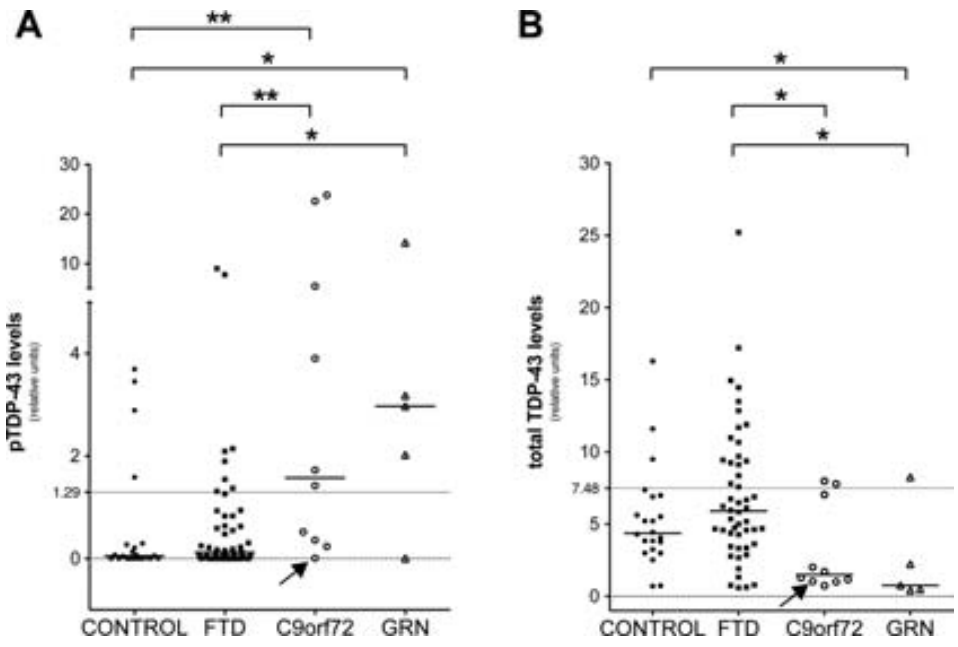


Figure 1 Plasma pTDP-43 and total TDP-43 levels in control subjects, patients with frontotemporal dementia (FTD) without mutations and subjects with the *C9orf72* repeat expansion or *GRN* mutations. (A) pTDP-43 levels are higher in *C9orf72* and *GRN* groups than in controls and FTD groups (p<0.05, Mann-Whitney). Significant differences between control versus *C9orf72* (p=0.002), control versus *GRN* (p=0.026), FTD versus *C9orf72* (p=0.004) and FTD versus *GRN* (p=0.022) groups. No differences were found between control versus FTD (p=0.204) and *C9orf72* versus *GRN* (p=0.903) groups. (B) Patients carrying *GRN* mutations had statistically significant decreased plasma total TDP-43 levels compared with controls and FTD groups (p=0.046 and p=0.015, respectively, Mann-Whitney). Patients carrying the *C9orf72* repeat expansion had statistically significant decreased plasma total TDP-43 levels compared with FTD group patients (p=0.016, Mann-Whitney) and a trend towards decreased levels compared with controls (p=0.096, Mann-Whitney). The short horizontal bars indicate the median pTDP-43 or total TDP-43 levels per group. The arrow corresponds to the asymptomatic subject carrying a *C9orf72* repeat expansion. The dashed horizontal line indicates the 99% upper confidence level for the control group. *p<0.05; **p<0.01.

clinical data and CSF AD biomarkers. pTDP-43 and total TDP-43 levels in CSF were not influenced by gender or age in the entire group or by the age of onset, disease duration, MMSE or GDS scores in FTD cases. CSF pTDP-43 levels in *C9orf72* expansion or *GRN* mutation carriers were slightly increased compared with the remaining patients with FTD (p=0.022 and p=0.014, respectively, Mann-Whitney; figure 2A, table 2) but not compared with controls (p=0.144 *C9orf72* vs control; p=0.094 *GRN* vs control). Four out of 5 (80%) mutation carriers were above the upper 99% CI of the control group compared with 2 out of 20 (10%) subjects in the FTD group. We also measured total TDP-43 levels in CSF samples and we did not find differences between groups (p=0.466, Kruskal-Wallis, figure 2B, table 2). No correlation was observed between pTDP-43 and total TDP-43 levels in CSF.

Association between plasma and CSF pTDP-43 levels

We next examined pTDP-43 levels in the subset of patients with FTD (n=20) for whom plasma and CSF were drawn at the same time point. Despite the reduced number of samples, plasma and CSF pTDP-43 levels correlated with each other (Spearman's r=+0.673, p=0.001, figure 3). However, plasma

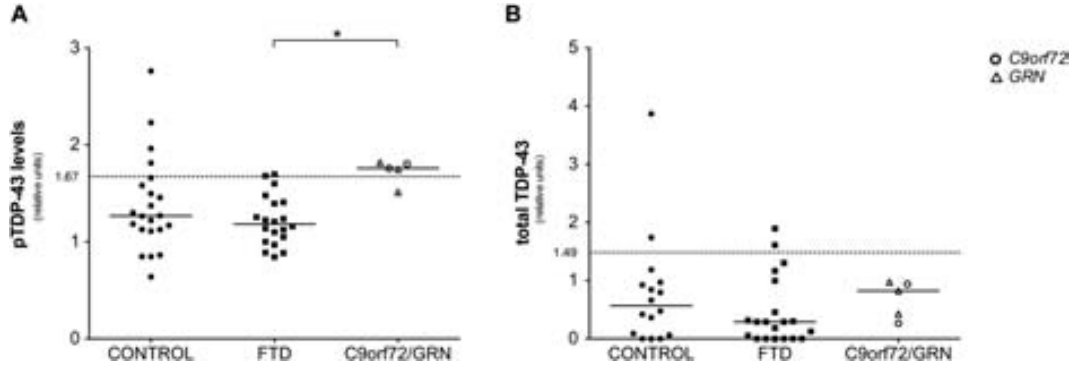


Figure 2 Cerebrospinal fluid pTDP-43 and total TDP-43 levels in control subjects and patients with frontotemporal dementia (FTD). (A) pTDP-43 levels in patients carrying the *C9orf72* expansion or *GRN* mutations are higher than those in patients with FTD without mutations (p=0.022 and p=0.014, respectively, Mann-Whitney). (B) No differences in total TDP-43 levels were found between groups. Patients with *C9orf72* (n=2) are depicted with a white circle and those with a *GRN* mutation (n=3) with a white triangle. The dashed horizontal line indicates the 99% upper confidence level for the control group. *p<0.05.

and CSF total TDP-43 levels did not correlate with each other (Spearman's r=+0.096, p=0.688, n=20).

DISCUSSION

The main finding in this study is that patients with FTD with the *C9orf72* repeat expansion or *GRN* mutations had higher plasma levels of pTDP-43 than patients with FTD without mutations or healthy controls. Conversely, subjects with the *C9orf72* repeat expansion or *GRN* mutations had lower plasma levels of total TDP-43 than patients with FTD without mutations. CSF pTDP-43 levels were also higher in patients with FTD with mutations than in those without mutations. Another relevant finding in our study is that plasma and CSF pTDP-43 levels correlated positively with each other, although plasma and CSF total TDP-43 did not.

The discovery of a reliable biomarker to detect FTLD-TDP is an essential step to perform an accurate diagnosis and ultimately to develop therapeutic strategies aimed at altering the natural course of the disease. This is particularly important in FTLD since this condition is known to be associated with several underlying pathologies that can present with the same clinical syndrome.[31] The search for biomarkers in neurodegenerative diseases over recent decades has yielded some positive results. The measurement of plasma and serum progranulin levels in FTD has proved to be a reliable biomarker to identify symptomatic and asymptomatic carriers of *GRN* mutations.[32–34] However, we have recently shown that plasma progranulin levels are not influenced by the *C9orf72* repeat expansion.[26] Progranulin probably represents the most reliable plasma marker in neurodegenerative diseases, but other promising biomarkers are being investigated. CSF analysis has shown to be useful in the diagnosis of AD. The pattern of reduced $A\beta_{42}$ and increased t-tau and p-tau levels accurately predicts AD pathology.[28 35] CSF examination can also be used to differentiate AD from FTLD with acceptable specificity.[36–38] However, in FTD, current CSF biomarkers are more useful to exclude AD than to confirm the pathophysiological process characteristic of FTLD. In our study, we only included cases without an AD CSF profile that could have biased our sample. Some other CSF biomarkers that have been tested in FTLD yielded variable results.[17] There is, therefore, a great need to develop markers to diagnose FTLD, detect the underlying pathology in vivo, and eventually better guide the future application of specific disease-modifying therapies.

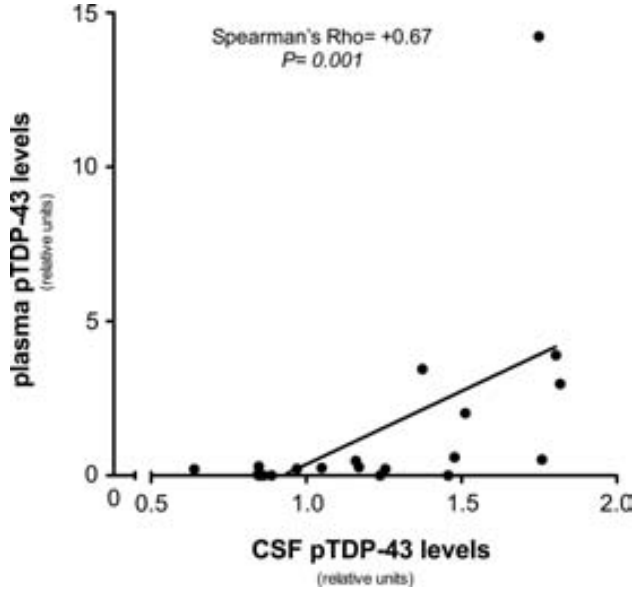


Figure 3 Correlation between plasma and cerebrospinal fluid (CSF) pTDP-43 levels. The y-axis represents plasma pTDP-43 and the x-axis represents CSF pTDP-43. A positive correlation between plasma and CSF levels was detected (Spearman's r=+0.673, p=0.001). Plasma and CSF samples of each patient were obtained at the same time.

TDP-43 is a 414-aminoacid protein that is usually located in the cell nucleus. It belongs to the heterogeneous ribonucleoprotein family and it presumably participates in several cell functions such as DNA transcription, mRNA splicing, mRNA export/import or miRNA synthesis and regulation.[39] TDP-43 inclusions in the brain and/or spinal cord in FTLD consist of abnormally aggregated, ubiquitinated and hyperphosphorylated TDP-43.[13 14] In particular, the importance of phosphorylation of the serine residues S409/410 of TDP-43 has been highlighted as a consistent feature in pathological inclusions of all TDP-43 proteinopathies.[40]

Previous studies have measured plasma TDP-43 and pTDP-43 in FTD.[18 19] They mainly found that levels of TDP-43 (total and pTDP-43) in plasma are elevated in subjects with FTLD-TDP. More importantly, they demonstrated a good correlation between plasma pTDP-43 levels and brain deposition of pTDP-43.[19] These results suggest that plasma pTDP43 levels could represent a good in vivo biomarker of TDP-43 pathology.

In this study, we found that subjects carrying a repeat expansion in *C9orf72* or a *GRN* mutation had significantly higher plasma pTDP-43 levels than those from control subjects and the remaining patients with FTD. In contrast, total TDP-43 levels in plasma were decreased in subjects carrying a repeat expansion in *C9orf72* or a *GRN* mutation. Three different hypotheses can be postulated to explain these results. First, since *C9orf72* expansion and *GRN* mutation carriers are known to be FTLD-TDP and plasma pTDP-43 correlates with brain pTDP-43 deposition,[19] higher levels of plasma pTDP-43 may consequently reflect an increase in brain pTDP-43 burden compared with the remaining FTD cases (in whom other underlying pathologies such us FTLD-tau or FTLD-FUS may be present). Second, as we found a correlation between age and plasma TDP-43 levels, higher levels of pTDP-43 in younger subjects may be the result of age rather than mutation status or brain pathology. This seems unlikely, however, since our results remained significant after adjusting for age. Finally, the *C9orf72* expansion and *GRN* mutations could elevate directly or indirectly pTDP-43 levels regardless of the burden of brain deposition of pTDP-43. Our data favour this last hypothesis and suggest that phosphorylated and non-phosphorylated forms of TDP-43 are in equilibrium as they correlate inversely in plasma. It could be speculated that the *C9orf72* expansion or mutations in *GRN* alter the balance between phosphorylated and non-phosphorylated forms of TDP, promoting the former one. This imbalance would consequently explain the increase of plasma pTDP-43 together with the decrease in plasma total TDP-43 levels found in subjects with the *C9orf72* expansion or mutations in *GRN*. Further studies are needed to explain the lack of correlation between pTDP-43 and total TDP-43 levels in the CSF and to explore why levels of CSF total TDP-43 in subjects with the *C9orf72* expansion or mutations in *GRN* are similar to those in other groups.

Our results in patients with FTD without mutations differ from previous studies that have examined TDP-43 in patients with FTD. Two studies in particular have shown an increase in total TDP-43 levels in plasma and CSF in FTD cases compared with controls.[18 21] Although in our study the levels of total TDP-43 in plasma were slightly increased in FTD compared with controls, none of the comparisons were significant. Differences in the assays used, sample size and study design may explain the discrepancies among findings.

Our study also addresses, for the first time, the relationship between levels of pTDP43 and total TDP-43 in plasma and CSF in FTD cases and controls. The values obtained in CSF were far more homogenous that those in plasma and we did not find differences in pTDP-43 or total TDP-43 levels between FTD cases and controls. The only significant finding was an increase in pTDP-43 levels in subjects with *C9orf72* or *GRN* mutations compared with the remaining patients with FTD. These results should be interpreted with caution due to the low number of patients with the *C9orf72* expansion (n=2) and *GRN* mutations (n=3) and the fact that most values in the whole FTD group were below the upper 99% CI limit of the control group. Of interest, CSF and plasma pTDP-43, but not total TDP-43, correlated in patients for whom both samples were available (n=20). This finding would suggest that plasma and CSF pTDP-43 levels are in equilibrium and would support the role of plasma as a reliable source of biochemical signatures. However, the lack of correlation between total TDP-43 in plasma and CSF remains unclear and deserves further investigation.

The strengths of this study are the inclusion of a well-characterised patient sample of mutation carriers, the use of CSF biomarkers to exclude AD, and the simultaneous measure of plasma and CSF in some patients. Nevertheless, this study has some limitations. First, the reduced sample size may have limited the power to detect differences, especially regarding CSF measurements. Second, the lack of neuropathological confirmation may have biased our results. Finally, the ELISA assays used show large interassay variability, particularly when using plasma samples. This high variability and the overlap among groups in plasma pTDP-43 levels may preclude the application of this assay in clinical settings. Conversely, CSF values were more homogenous and the interassay variability was lower. We acknowledge that these results are preliminary and should be confirmed in larger samples, preferably with pathologically or genetically confirmed cases, and with the use of other assays. To further determine whether pTDP-43 can be used as a reliable plasma biomarker for FTLD-TDP, patients with the *C9orf72* expansion or *GRN* mutations should be compared with patients with mutations in *MAPT*, known to cause FTLD-tau.

In conclusion, our results suggest that plasma pTDP-43 levels are increased in patients carrying the *C9orf72* repeat expansion or *GRN* mutations, conditions associated with TDP-43 proteinopathies. If confirmed, pTDP-43 levels may represent a reliable plasma biomarker for FTLD-TDP.

Author affiliations
[1]Neurology Department, Hospital de la Santa Creu i Sant Pau, Universitat Autònoma de Barcelona, Barcelona, Spain
[2]Center for Networker Biomedical Research in Neurodegenerative Diseases (CIBERNED), Madrid, Spain
[3]Department of Neurology, Alzheimer's Disease and other Cognitive Disorders Unit, Hospital Clínic, Barcelona, Spain
[4]Memory Clinic of Fundació ACE, Institut Català de Neurociències Aplicades, Barcelona, Spain
[5]Neurology Department, Hospital Universitari Son Espases, Majorca, Spain
[6]Institut de Neuropatología, Servei Anatomia Patológica, IDIBELL, Hospital Universitari de Bellvitge, University of Barcelona, Barcelona, Spain
[7]Department of Molecular Genetics, Neurodegenerative Brain Disease Group, VIB, Antwerp, Belgium
[8]Laboratory of Neurogenetics, Institute Born-Bunge, University of Antwerp, Antwerp, Belgium

Acknowledgements The authors thank all the patients and their families for their participation. They thank Carolyn Newey for editorial help, and Laia Muñoz and Inés Matas for technical assistance.

Contributors All authors are justifiably credited with authorship, according to the authorship criteria. In detail: MS-C—conception, design, analysis and interpretation of data, drafting of the manuscript, critical revision of manuscript, final approval given; OD-I—analysis and interpretation of data, critical revision of manuscript, final approval given; AL—analysis and interpretation of data, critical revision of manuscript, final approval given; RS-V—analysis and interpretation of data, critical revision of manuscript, final approval given; IH—analysis and interpretation of data, critical revision of manuscript, final approval given; GA—analysis and interpretation of data, critical revision of manuscript, final approval given; SA-A—analysis and interpretation of data, critical revision of manuscript, final approval given; DA—analysis and interpretation of data, critical revision of manuscript, final approval given; JF—analysis and interpretation of data, critical revision of manuscript, final approval given; IF—critical revision of manuscript, final approval given; JvdZ—analysis and interpretation of data, critical revision of manuscript, final approval given; LD—analysis and interpretation of data, critical revision of manuscript, final approval given; CVB—critical revision of manuscript, final approval given; JLM—critical revision of manuscript, final approval given; RB—critical revision of manuscript, final approval given; JC—design, analysis and interpretation of data, critical revision of manuscript, final approval given; AL—conception, design, analysis and interpretation of data, drafting of the manuscript, final approval given.

Funding This work was supported by a research grant from 'Centro de Investigación en Red en Enfermedades Neurodegenerativas' and by the 'Fondo de Investigaciones Sanitarias' (PI09/00098). At the Antwerp site, the genetic screening of *C9orf72* for the repeat expansion was funded in part by the Interuniversity Attraction Poles program of the Belgian Science Policy Office; the Methusalem Excellence program of the Flemish Government; the Medical Foundation Queen Elisabeth, the Research Foundation Flanders (FWO), Belgium The FWO provided a postdoctoral fellowship to JvdZ.

Competing interests None.

Ethics approval The 'Hospital de la Santa Creu i Sant Pau' ethical standards committee on human experimentation approved the study. Barcelona, Spain (code: 5/2010).

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

1 Kumar-Singh S, Van Broeckhoven C. Frontotemporal lobar degeneration: current concepts in the light of recent advances. *Brain Pathol* 2007;17:104–14.
2 Neary D, Snowden JS, Gustafson L, *et al*. Frontotemporal lobar degeneration: a consensus on clinical diagnostic criteria. *Neurology* 1998;51:1546–54.
3 Van Langenhove T, Van der Zee J, Van Broeckhoven C, *et al*. The molecular basis of the frontotemporal lobar degeneration-amyotrophic lateral sclerosis spectrum. *Ann Med* 2012;44:817–28.
4 Mackenzie IR, Neumann M, Bigio EH, *et al*. Nomenclature and nosology for neuropathologic subtypes of frontotemporal lobar degeneration: an update. *Acta Neuropathol* 2010;119:1–4.
5 Stevens M, Van Duijn CM, Kamphorst W, *et al*. Familial aggregation in frontotemporal dementia. *Neurology* 1998;50:1541–5.
6 Baker M, Mackenzie IR, Pickering-Brown SM, *et al*. Mutations in progranulin cause tau-negative frontotemporal dementia linked to chromosome 17. *Nature* 2006;442:916–9.
7 Cruts M, Gijselinck I, Van der Zee J, *et al*. Null mutations in progranulin cause ubiquitin-positive frontotemporal dementia linked to chromosome 17q21. *Nature* 2006;442:920–4.
8 Dumanchin C, Camuzat A, Campion D, *et al*. Segregation of a missense mutation in the microtubule-associated protein tau gene with familial frontotemporal dementia and parkinsonism. *Hum Mol Genet* 1998;7:1825–9.
9 DeJesus-Hernandez M, Mackenzie IR, Boeve BF, *et al*. Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. *Neuron* 2011;72:245–56.
10 Gijselinck I, Van Langenhove T, Van der Zee J, *et al*. A C9orf72 promoter repeat expansion in a Flanders-Belgian cohort with disorders of the frontotemporal lobar degeneration-amyotrophic lateral sclerosis spectrum: a gene identification study. *Lancet Neurol* 2012;11:54–65.
11 Renton AE, Majounie E, Waite A, *et al*. A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. *Neuron* 2011;72:257–68.
12 Van der Zee J, Gijselinck I, Dillen L, *et al*. A pan-European study of the C9orf72 repeat associated with FTLD: geographic prevalence, genomic instability, and intermediate repeats. *Hum Mutat* 2013;34:363–73.
13 Arai T, Hasegawa M, Akiyama H, *et al*. TDP-43 is a component of ubiquitin-positive tau-negative inclusions in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Biochem Biophys Res Commun* 2006;351:602–11.
14 Neumann M, Sampathu DM, Kwong LK, *et al*. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Science* 2006;314:130–3.
15 Benajiba L, Le Ber I, Camuzat A, *et al*. TARDBP mutations in motoneuron disease with frontotemporal lobar degeneration. *Ann Neurol* 2009;65:470–3.
16 Sreedharan J, Blair IP, Tripathi VB, *et al*. TDP-43 mutations in familial and sporadic amyotrophic lateral sclerosis. *Science* 2008;319:1668–72.

17 Hu WT, Trojanowski JQ, Shaw LM. Biomarkers in frontotemporal lobar degenerations–progress and challenges. *Prog Neurobiol* 2011;95:636–48.
18 Foulds P, McAuley E, Gibbons L, *et al*. TDP-43 protein in plasma may index TDP-43 brain pathology in Alzheimer's disease and frontotemporal lobar degeneration. *Acta Neuropathol* 2008;116:141–6.
19 Foulds PG, Davidson Y, Mishra M, *et al*. Plasma phosphorylated-TDP-43 protein levels correlate with brain pathology in frontotemporal lobar degeneration. *Acta Neuropathol* 2009;118:647–58.
20 Kasai T, Tokuda T, Ishigami N, *et al*. Increased TDP-43 protein in cerebrospinal fluid of patients with amyotrophic lateral sclerosis. *Acta Neuropathol* 2009;117:55–62.
21 Steinacker P, Hendrich C, Sperfeld AD, *et al*. TDP-43 in cerebrospinal fluid of patients with frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Arch Neurol* 2008;65:1481–7.
22 Fortea J, Lladó A, Clarimón J, *et al*. PICOGEN: five years experience with a genetic counselling program for dementia. *Neurologia* 2011;26:143–9.
23 Rascovsky K, Hodges JR, Knopman D, *et al*. Sensitivity of revised diagnostic criteria for the behavioural variant of frontotemporal dementia. *Brain* 2011;134:2456–77.
24 Gorno-Tempini ML, Hillis A, Weintraub S, *et al*. Classification of primary progressive aphasia and its variants. *Neurology* 2011;76:1006–14.
25 Antonell A, Gil S, Sánchez-Valle R, *et al*. Serum progranulin levels in patients with frontotemporal lobar degeneration and Alzheimer's disease: detection of GRN mutations in a Spanish cohort. *J Alzheimers Dis* 2012;31:581–91.
26 Dols-Icardo O, Suárez-Calvet M, Hernández I, *et al*. Expansion mutation in C9ORF72 does not influence plasma progranulin levels in frontotemporal dementia. *Neurobiol Aging* 2012;33:1851.e17–19.
27 Costa S, Suárez-Calvet M, Antón S, *et al*. Comparison of 2 diagnostic criteria for the behavioral variant of frontotemporal dementia. *Am J Alzheimers Dis Other Demen* 2013;28:469–76.
28 Mattsson N, Zetterberg H, Hansson O, *et al*. CSF biomarkers and incipient Alzheimer disease in patients with mild cognitive impairment. *JAMA* 2009;302:385–93.
29 Fukumoto H, Rosene DL, Moss MB, *et al*. Beta-secretase activity increases with aging in human, monkey, and mouse brain. *Am J Pathol* 2004;164:719–25.
30 Dols-Icardo O, García-Redondo A, Rojas-García R, *et al*. Characterization of the repeat expansion size in C9orf72 in amyotrophic lateral sclerosis and frontotemporal dementia. *Hum Mol Genet* Published Online First: 20 September 2013. doi: 10.1093/hmg/ddt460
31 Josephs KA, Hodges JR, Snowden JS, *et al*. Neuropathological background of phenotypical variability in frontotemporal dementia. *Acta Neuropathol* 2011;122:137–53.
32 Finch N, Baker M, Crook R, *et al*. Plasma progranulin levels predict progranulin mutation status in frontotemporal dementia patients and asymptomatic family members. *Brain* 2009;132:583–91.
33 Ghidoni R, Benussi L, Glionna M, *et al*. Low plasma progranulin levels predict progranulin mutations in frontotemporal lobar degeneration. *Neurology* 2008;71:1235–9.
34 Sleegers K, Brouwers N, Van Damme P, *et al*. Serum biomarker for progranulin-associated frontotemporal lobar degeneration. *Ann Neurol* 2009;65:603–9.
35 Blennow K, Hampel H, Weiner M, *et al*. Cerebrospinal fluid and plasma biomarkers in Alzheimer disease. *Nat Rev Neurol* 2010;6:131–44.
36 Bian H, Van Swieten JC, Leight S, *et al*. CSF biomarkers in frontotemporal lobar degeneration with known pathology. *Neurology* 2008;70:1827–35.
37 Bibl M, Gallus M, Welge V, *et al*. Cerebrospinal fluid amyloid-β 2-42 is decreased in Alzheimer's, but not in frontotemporal dementia. *J Neural Transm* 2012;119:805–13.
38 Kapaki E, Paraskevas GP, Papageorgiou SG, *et al*. Diagnostic value of CSF biomarker profile in frontotemporal lobar degeneration. *Alzheimer Dis Assoc Disord* 2008;22:47–53.
39 Buratti E, Baralle FE. The multiple roles of TDP-43 in pre-mRNA processing and gene expression regulation. *RNA Biol* 2010;7:420–9.
40 Neumann M, Kwong LK, Lee EB, *et al*. Phosphorylation of S409/410 of TDP-43 is a consistent feature in all sporadic and familial forms of TDP-43 proteinopathies. *Acta Neuropathol* 2009;117:137–49.

Plasma phosphorylated TDP-43 levels are elevated in patients with frontotemporal dementia carrying a *C9orf72* repeat expansion or a *GRN* mutation

Marc Suárez-Calvet, Oriol Dols-Icardo, Albert Lladó, et al.

J Neurol Neurosurg Psychiatry published online December 4, 2013
doi: 10.1136/jnnp-2013-305972

HMG Advance Access published October 8, 2013

Human Molecular Genetics, 2013 **1–6**
doi:10.1093/hmg/ddt460

Characterization of the repeat expansion size in *C9orf72* in amyotrophic lateral sclerosis and frontotemporal dementia

Oriol Dols-Icardo[1,2], Alberto García-Redondo[3,4], Ricard Rojas-García[1,2], Raquel Sánchez-Valle[5], Aina Noguera[6], Estrella Gómez-Tortosa[7], Pau Pastor[2,8,9], Isabel Hernández[10], Jesús Esteban-Pérez[3,4], Marc Suárez-Calvet[1,2], Sofía Antón-Aguirre[1,2], Guillermo Amer[11], Sara Ortega-Cubero[2,8,9], Rafael Blesa[1,2], Juan Fortea[1,2], Daniel Alcolea[1,2], Aura Capdevila[1], Anna Antonell[5], Albert Lladó[5], José Luís Muñoz-Blanco[12], Jesús S. Mora[13], Lucía Galán-Dávila[14], Francisco Javier Rodríguez De Rivera[15], Alberto Lleó[1,2] and Jordi Clarimón[1,2,*]

[1]Memory Unit and Neuromuscular Diseases Unit, Neurology Department and Sant Pau Biomedical Research Institute, Hospital de la Santa Creu i Sant Pau, Universitat Autònoma de Barcelona, Barcelona, Spain [2]Center for Networking Biomedical Research in Neurodegenerative Diseases (CIBERNED), Madrid, Spain [3]Department of Neurology, ALS Unit, Instituto de Investigación Biomédica Hospital 12 de Octubre, Madrid, Spain [4]Centre for Biomedical Network Research on Rare Diseases (CIBERER), Valencia, Spain [5]Department of Neurology, Alzheimer's Disease and Other Cognitive Disorders Unit, Institute of Neurosciences, IDIBAPS, Hospital Clínic, Barcelona, Spain [6]Department of Clinical Analysis, Hospital Universitari Son Espases, Mallorca, Spain [7]Department of Neurology, Fundación Jiménez-Díaz, Madrid, Spain [8]Neurogenetics Laboratory, Division of Neurosciences, Center for Applied Medical Research, University of Navarra, Pamplona, Spain [9]Department of Neurology, Clínica Universidad de Navarra, University of Navarra School of Medicine, Pamplona, Spain [10]Memory Clinic of Fundació ACE, Institut Català de Neurociències Aplicades, Barcelona, Spain [11]Department of Neurology, Hospital Universitari Son Espases, Mallorca, Spain [12]Department of Neurology, ALS Unit, Hospital Universitario Gregorio Marañón, Madrid, Spain [13]Department of Neurology, ALS Unit, Hospital Carlos III, Madrid, Spain [14]Department of Neurology, ALS Unit, Hospital Clínico Universitario 'San Carlos', Madrid, Spain [15]Department of Neurology, ALS Unit, Hospital Universitario La Paz, Madrid, Spain

Received August 27, 2013; Revised and Accepted September 16, 2013

Hexanucleotide repeat expansions within the *C9orf72* gene are the most important genetic cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). The difficulty of developing a precise method to determine the expansion size has hampered the study of possible correlations between the hexanucleotide repeat number and clinical phenotype. Here we characterize, through a new non-radioactive Southern blot protocol, the expansion size range in a series of 38 ALS and 22 FTD heterozygous carriers of >30 copies of the repeat. Maximum, median and modal hexanucleotide repeat number were higher in ALS patients than in FTD patients ($P < 0.05$ in all comparisons). A higher median number of repeats correlated with a bigger range of repeat sizes (Spearman's $\rho = 0.743$, $P = 1.05 \times 10^{-11}$). We did not find any correlation between age of onset or disease duration with the repeat size in neither ALS nor FTD mutation carriers. Clinical presentation (bulbar or spinal) in ALS patients did not correlate either with the repeat length. We finally analyzed two families with affected and unaffected repeat expansion carriers, compared the size of the repeat expansion between two monozygotic (MZ) twins (one affected of ALS and the other unaffected), and examined the expansion size in two different tissues (cerebellum and peripheral blood) belonging to the same FTD patient. The results suggested that the length

*To whom correspondence should be addressed at: Genetics of Neurodegenerative Disorders Unit, IIB-Sant Pau, Neurology Department, Hospital de la Santa Creu i Sant Pau. Sant Antoni M. Claret 167, 08025 Barcelona, Spain. Tel: +34 932919050 x8240; Fax: +34 935565602; Email: jclarimon@santpau.cat

of the *C9orf72* repeat varies between family members, including MZ twins, and among different tissues from the same individual.

INTRODUCTION

A non-coding (GGGGCC) hexanucleotide repeat expansion within the first intron of the *chromosome 9 open reading frame 72* (*C9orf72*) gene (GenBank reference NM_001256054.1; MIM# 614260) has been identified as the most frequent cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) (1–3). Typically, a standard repeat-primed PCR (rpPCR) method is used to identify expansion carriers, but this technique cannot size genomic DNA expansions beyond 30 hexanucleotide repeats and therefore is ineffective to estimate larger repeat lengths of this dynamic mutation (1,2). Recently, the expansion size was characterized by Southern blotting through a non-radioactive approach using a hybridizing probe composed of five hexanucleotide repeats (4). This genotyping approach allowed the detection of expansions >275 repeats and showed that DNA isolated from lymphoblast cell lines is not reliable for sizing the *C9orf72* expansion. Comparisons of the repeat length across six disease cohorts from UK, including 11 peripheral blood (PB) specimens from FTD patients and 17 PB samples from ALS patients, did not reveal any significant differences (4). A Southern blot protocol using a radioactive probe has also been recently published, but the study did not include any phenotype correlation analysis (5).

In the present study, we show an optimized protocol for Southern blot hybridization using a 954-bp non-radioactive probe that is capable to accurately size the whole range of the *C9orf72* hexanucleotide repeat expansion. We also characterized pathogenic expansions through Southern blotting in a series of ALS and FTD patients, and assessed for possible correlations between the repeat expansion length and clinical features of these two neurodegenerative disorders.

RESULTS

Table 1 shows the demographic and clinical characteristics of the analyzed index patients. No differences in the proportion of gender or in the age of onset were found between ALS and FTD patients. In order to allay concerns that our Southern blot protocol might identify nonspecific bands, we first assessed eight DNA samples that did not carry the expansion mutation (Supplementary Material, Fig. S1). The resulting blot clearly demonstrated that our protocol did not detect any unexpected signals above the number of repeats determined by rpPCR genotyping. As presented in the Supplementary Material, Fig. S1, the protocol also showed a high sensitivity for the discrimination of low copy, non-pathogenic number of repeats (see control samples in Lanes VII and VIII carrying 7/25 and 2/19 repeats, respectively). All cases with a pathological repeat expansion in one allele resulted in higher molecular weight signals on the membrane, thus demonstrating the high specificity of the Southern blot protocol (Fig. 1). This pattern was seen in genomic DNA extracted from PB ($N = 64$) and cerebellum ($N = 5$).

Table 1. Demographic, clinical and C9orf72 hexanucleotide repeat number comparisons between ALS and FTD index patients

	ALS ($n = 38$)	FTD ($n = 22$)	*P*-value
Gender (% female)	47.4	50	1
Age at onset (mean years ± SD)	55.42 ± 8.7	54.45 ± 7.1	0.591
Positive family history (%)	60	66.7	0.777
Site of onset (bulbar/spinal)	13/25	NA	NA
Hexanucleotide repeat number			
Median ± SD	1642 ± 607	1291 ± 637	0.020
Minimum (median ± SD)	1082 ± 427	916 ± 475	0.090
Maximum (median ± SD)	2245 ± 872	1666 ± 838	0.012
Mode (median ± SD)	1849 ± 808	1338 ± 671	0.010

Figure 2 shows the estimation of hexanucleotide repeat number and size range for all expansion mutation carriers. For statistical analyses, we considered four different metrics of the repeat length that included minimum, maximum, median and modal number of repeats for those patients in which a dense smear fragment appeared beyond the average allele length. None of these metrics correlated with gender or family history of disease in either the entire cohort or the two clinical phenotypes, independently. Likewise, the four expansion size measures did not correlate with age of clinical onset or disease duration (from onset to death) in FTD nor in ALS patients (data not shown). Of note, ALS patients had larger expansions than FTD patients, with significantly higher maximum, median and modal number of hexanucleotide repeats ($P = 0.012$, 0.02 and 0.01, respectively; Table 1). None of the four estimates were different between ALS cases with bulbar or spinal onset (i.e. median, 1693 ± 755 versus 1615 ± 530, respectively, $P = 0.377$). The median hexanucleotide repeat number significantly correlated with the range of repeat sizes (Spearman's $\rho = 0.743$, $P = 1.05 \times 10^{-11}$; Supplementary Material, Fig. S2), thus indicating that larger expansions presented greater hexanucleotide repeat ranges.

The expansion size was then determined in a family in which the mutation segregated with FTD (Supplementary Material, Fig. S3A). The three affected siblings had a median repeat expansion size of 143, and ranged from 128 to 154. However, expansion mutation carriers from the second generation, composed of two asymptomatic first cousins, carried a median of 120 and 1401 repeats. Besides, the repeat expansion was also assessed in an ALS patient, who started the disease at the age of 57 years old, and his three unaffected sons (two males and one women) who were also mutation carriers (Supplementary Material, Fig. S3B). In this case, the father presented a median of 1898 hexanucleotide repeats, which was slightly larger to the number presented by the three siblings (1516, 1451 and 1127 repeats).

We also compared the repeat expansion size between a 50-year-old index ALS patient with symptoms beginning at the age of 47, and his unaffected MZ twin. All hexanucleotide repeats metrics were higher in the affected ALS patient compared with his sibling (Supplementary Material, Table S1).

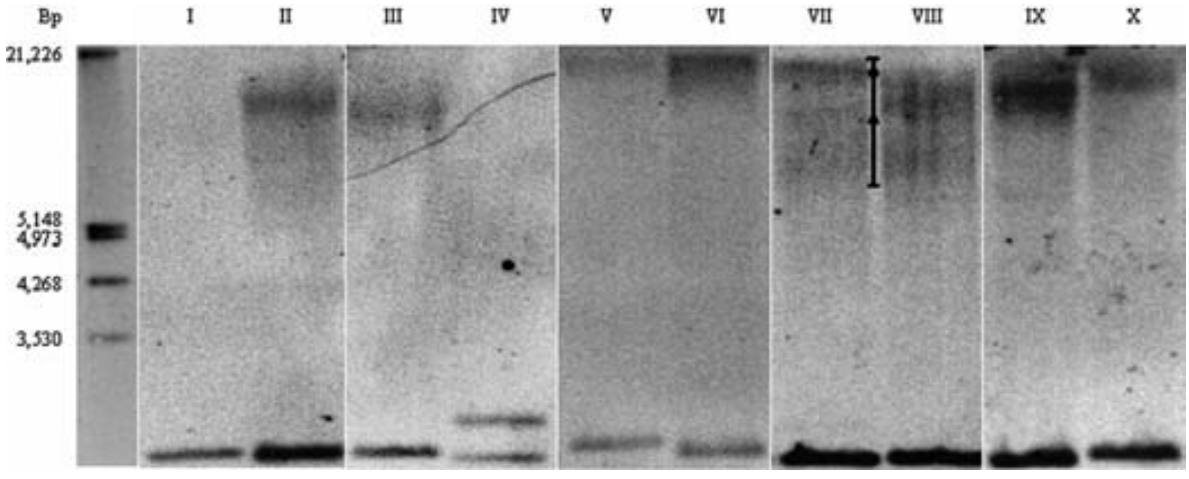


Figure 1. Representative Southern blots of small and large repeat expansions. Nine cases carrying repeat expansion (Lanes 2–10) and a control DNA without an expansion (first lane) are shown. Lane 7 (corresponding to case 46 in Fig. 2) shows an example of median and mode determination, represented with a triangle and a round, respectively, within the smear range.

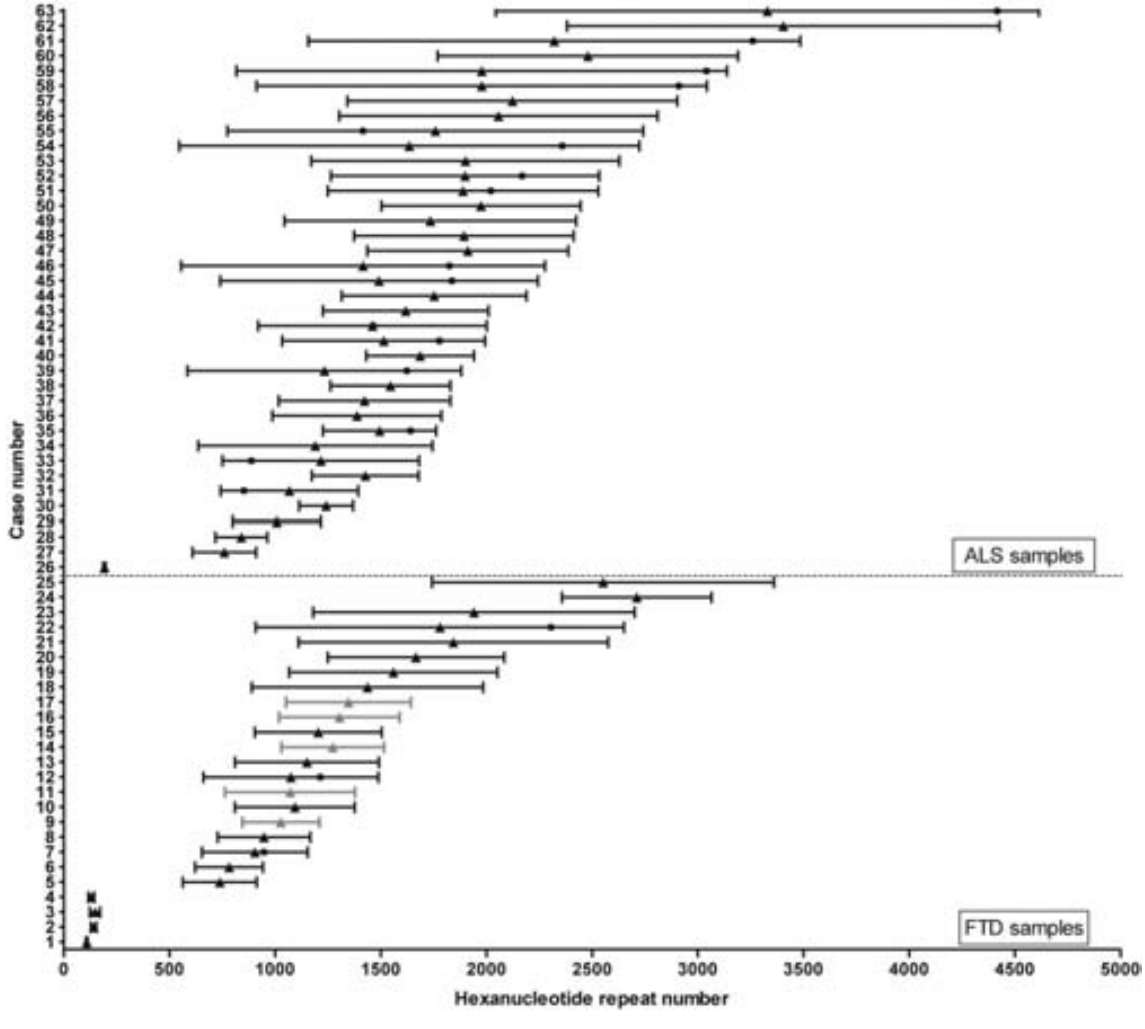


Figure 2. Representation of Southern blot data for ALS ($n = 38$) and FTD ($n = 22$) patients. Individual blot data are shown with approximated hexanucleotide repeat number range (minimum to maximum), median represented with a triangle and modal point as a black dot. DNA samples extracted from PB tissue and cerebellum tissue are represented with a black or gray line, respectively. Cases 13 and 14 represent repeat expansion size parameters of PB and cerebellum tissue from the same individual, respectively (Case 14 is not included in the statistical analysis). Note that Cases 1 and 2 are FTD patients belonging to the FTD family (individuals II and III from the FTD family pedigree represented in Supplementary Material, Fig. S3A) and are not included for statistical purposes.

Finally, the hexanucleotide repeat number was determined in DNA samples from cerebellum and PB tissue belonging to the same FTD patient. Expansion size estimates showed a moderately higher number of hexanucleotide repeats in the cerebellum compared with PB tissue (Supplementary Material, Table S1).

DISCUSSION

We have developed a non-radioactive Southern blotting protocol using a 954-bp probe to characterize the hexanucleotide repeat expansion size range in a series of ALS and FTD patients (Supplementary Material, Fig. S4). Our method allows the detection of hexanucleotide repeats in *C9orf72* ranging from 2 to ∼4500 repeat units, thus covering the whole expansion size range and enabling the identification of both small and large pathogenic repeat expansions. Our results suggest that ALS patients harbour a higher number of hexanucleotide repeats than FTD patients. This is not in accordance with a recent report in which authors did not find repeat size differences between different neurodegenerative diseases, including ALS and FTD (4). One possibility for this discrepancy is that their methodology, based on a hybridization probe composed of five DIG-labelled hexanucleotide repeats, was not sensitive to detect expansions at the low edge of the mutation spectrum (from 30 to ∼ 275 repeats), and therefore DNA samples with low number of repeats could have been missed. This outcome clearly emphasizes the need of a genotyping technique with enough sensitivity to cover the whole pathogenic expansion size range. Another explanation could be that we have analyzed a greater number of ALS and FTD patients, thus increasing our power to detect differences between both neurodegenerative diseases.

A possible mechanistic explanation for the difference in the repeat length between ALS and FTD in our study might be related to the recently described repeat-associated non-ATG-initiated (RAN) translation that occurs in *C9orf72* expansion mutation carriers. This alteration in the translation process leads to the accumulation of insoluble dipeptide-repeat (DPR) protein aggregates in the central nervous system (6,7). Interestingly, the formation of incorrectly translated products seems to occur in a hexanucleotide repeat length-dependent manner. That is, the longer the repeat size the greater quantity of aberrantly translated dipeptides will be produced. Furthermore, longer repeat tracts can express multiple RAN-translated homopolymeric proteins whereas shorter repeats only express a unique DPR (7). Taking all these novel data into account, it could be expected that the length of the repeat could determine, to some extent, the molecular mechanisms driving the disease to either FTD or ALS. Nevertheless, our results should be interpreted cautiously as there is a substantial overlap of hexanucleotide repeat sizes between ALS and FTD index patients, and a threshold to differentiate between ALS and FTD cannot be defined based on our data. For example, index cases number 3, 4 and 26 (Fig. 2), with estimated median allele sizes of 148, 130 and 192 repeats, suffered from either FTD (Patients 3 and 4) or ALS (Patient 26).

Our results also suggest that the hexanucleotide repeat number does not correlate with disease duration or age at onset. This lack of correlation might be due to environmental, genetic or epigenetic factors. In this sense, a recent study has reported that higher levels of DNA methylation near the hexanucleotide repeat significantly correlated with shorter disease duration in ALS patients (8).

We found a striking positive correlation between the median number of hexanucleotide repeats and the range of the observed repeat sizes. This might reflect a greater disruption of replication, repair and/or recombination machineries as the number of repeats increases, thus contributing to genetic instability (9). We also show a family in which the expansion mutation segregates with FTD and present evidence of an unstable hexanucleotide repeat number transference through generations. On the contrary, we also present a family with an ALS affected father and his three healthy sons who carry similar, although slightly lower, expansion sizes. Similar expansion sizes (ranging from 1000 to 1600 repeats) have been previously reported across four family members suffering from FTD (10). These data could explain the clinical heterogeneity between family members that has been described in related individuals carrying this dynamic mutation (11), and clearly indicates the impossibility to predict the expansion size carried by descendants of mutation carriers.

Finally, the fact that we have found subtle discordances in the repeat length between two tissues (PB and cerebellum) from the same patient, and also between MZ twins discordant for the disease, strongly suggests that stochastic expansion events during cell division, which results in somatic and/or germline mosaicism, contributes to the intra- and inter-individual repeat expansion variation.

In conclusion, we have developed a reproducible and optimized protocol of Southern blot hybridization that allows a reliable method to approximate the whole repeat expansion size range in *C9orf72* in DNA samples extracted from PB and brain tissue. Our results suggest that larger expansions occur in ALS compared with index FTD patients. We also demonstrate that inter-individual differences in repeat lengths might exist not only between unrelated patients but also between family members, including MZ twins, and different tissues from the same individual. These results could explain the high heterogeneity of the phenotype presented by patients carrying the *C9orf72* expansion.

MATERIALS AND METHODS

Samples

DNA samples extracted from PB of 38 index ALS patients and 18 index FTD patients were included in the analysis. Additionally, DNA samples extracted from cerebellum tissue of four patients with pathologically confirmed frontotemporal lobar degeneration were collected. PB-derived DNA samples of two families, one consisting of three FTD affected siblings and two non-affected first cousins, and the other composed of one ALS patient and his four unaffected sons, were also recruited. DNA from the cerebellum of an index FTD patient was also analyzed in order to compare the expansion size with its corresponding PB tissue. Finally, we included DNA from an unaffected monozygotic (MZ) twin of an index ALS patient in order to compare the number of hexanucleotide repeats between identical twins. ALS patients were diagnosed with definite or probable ALS, as defined by the El Escorial research criteria (12). FTD

diagnoses were made according to the international consensus criteria (13,14). All patients were previously identified as heterozygous carriers of a *C9orf72* hexanucleotide expansion >30 repeats through an rpPCR method (10,15–18). Written informed consent was obtained from all participants and research ethics committees from the respective participating centres approved the study.

Southern blotting

A total of 15 μg of gDNA was digested overnight with *Xba*I restriction enzyme (New England Biolabs, Ipswich, MA, USA). Electrophoresis of digested DNA samples was carried out in 0.8% agarose gel with 1× tris–borate–EDTA buffer for 22 h at 1.4 V/cm. DNA was transferred to a positively charged nylon membrane (Roche Applied Science) by capillary blotting and was baked at 80°C for 2 h. A 954-bp probe was synthesized through a nested PCR method with FastStart PCR Master Mix (Roche Applied Science) using two PCR rounds and four oligonucleotide primers. The first PCR round was performed with the primers CAACTGGTGAGTGATGGTGGTAG and CTGAG TTCCAGAGCTTGCTACAG. The 1350 bp PCR product was then purified and used as a template for a second PCR using the FastStart PCR Master Mix (Roche Applied Science) and the oligonucleotides CAGAAGGTGTAGACGTTGAGAGC and CAGCGAGTACTGTGAGAGCAAG. Digoxigenin (DIG)-dUTP labelling was performed with 2.8 μl of the Vial 2, contained in the DIG probe synthesis kit (Roche Applied Science). Prehybridization was carried out at 48°C for 3 h and hybridization at 45°C overnight. Blots were then washed in 2× standard sodium citrate (SSC) and 0.1% sodium dodecyl sulphate (SDS) at room temperature for 10 min twice. High stringency washes (0.1× SSC and 0.1% SDS) were carried out at 68°C for 10 min five times. Ready-to-use CDP-*Star* (Roche Applied Science) was used as the chemiluminescent substrate through the transparency technique and signals were visualized on a KODAK Image Station 4000MM PRO (Carestream Health Inc., Rochester, NY, USA).

Assessment of the number of repeats

Hexanucleotide repeat size was estimated by interpolation with a plot of log 10 bp number against migration distance. Minimum, maximum, median and modal sizes of the repeat expansion were assessed and used for statistical analyses. DIG-labelled DNA molecular weight marker III (Roche Applied Science) was used as a ladder.

Statistical analyses

Chi-square analyses were performed for categorical data. Mann–Whitney test was used to compare disease phenotypes and clinical outcomes with minimum, maximum, median and modal size of the repeat expansion. Spearman's correlations were performed between the four expansion size parameters and the clinical features of the disease. Data were analyzed with the Statistical Package for Social Science v19.0 (SPSS Inc., Chicago, IL, USA).

SUPPLEMENTARY MATERIAL

Supplementary Material is available at *HMG* online.

ACKNOWLEDGEMENTS

We thank patients, their families and controls for their participation in the study. The authors thank the Neurological Tissue Bank of the Biobanc-Hospital Clínic-IDIBAPS (Barcelona) for providing human brain samples, and specially Dr. Ellen Gelpí for her valuable help.

Conflict of Interest statement. None declared.

FUNDING

This work was supported by grants from the Spanish Ministry of Economy and Competitiveness [grants number PI12/01311 and PI10/00092], CIBERNED and by the Foundation for Applied Medical Research (FIMA) to S.O.-C. and P.P.

REFERENCES

1. DeJesus-Hernandez, M., Mackenzie, I.R., Boeve, B.F., Boxer, A.L., Baker, M., Rutherford, N.J., Nicholson, A.M., Finch, N.A., Flynn, H., Adamson, J. *et al.* (2011) Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. *Neuron*, **72**, 245–256.
2. Renton, A.E., Majounie, E., Waite, A., Simón-Sánchez, J., Rollinson, S., Gibbs, J.R., Schymick, J.C., Laaksovirta, H., van Swieten, J.C., Myllykangas, L. *et al.* (2011) A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. *Neuron*, **72**, 257–268.
3. Gijselinck, I., Van Langenhove, T., van der Zee, J., Sleegers, K., Philtjens, S., Kleinberger, G., Janssens, J., Bettens, K., Van Cauwenberghe, C., Pereson, S. *et al.* (2012) A C9orf72 promoter repeat expansion in a Flanders-Belgian cohort with disorders of the frontotemporal lobar degeneration-amyotrophic lateral sclerosis spectrum: a gene identification study. *Lancet Neurol.*, **11**, 54–65.
4. Beck, J., Poulter, M., Hensman, D., Rohrer, J.D., Mahoney, C.J., Adamson, G., Campbell, T., Uphill, J., Borg, A., Fratta, P. *et al.* (2013) Large C9orf72 hexanucleotide repeat expansions are seen in multiple neurodegenerative syndromes and are more frequent than expected in the UK population. *Am. J. Hum. Genet.*, **92**, 345–353.
5. Buchman, V.L., Cooper-Knock, J., Connor-Robson, N., Higginbottom, A., Kirby, J., Razinskaya, O.D., Ninkina, N. and Shaw, P.J. (2013) Simultaneous and independent detection of C9ORF72 alleles with low and high number of GGGGCC repeats using an optimised protocol of Southern blot hybridisation. *Mol. Neurodegener.*, **8**, 12.
6. Ash, P.E., Bieniek, K.F., Gendron, T.F., Caulfield, T., Lin, W.L., Dejesus-Hernandez, M., van Blitterswijk, M.M., Jansen-West, K., Paul, J.W. 3rd, Rademakers, R. *et al.* (2013) Unconventional translation of C9ORF72 GGGGCC expansion generates insoluble polypeptides specific to c9FTD/ALS. *Neuron*, **77**, 639–646.
7. Mori, K., Weng, S.M., Arzberger, T., May, S., Rentzsch, K., Kremmer, E., Schmid, B., Kretzschmar, H.A., Cruts, M., Van Broeckhoven, C. *et al.* (2013) The C9orf72 GGGGCC repeat is translated into aggregating dipeptide-repeat proteins in FTLD/ALS. *Science*, **339**, 1335–1338.
8. Xi, Z., Zinman, L., Moreno, D., Schymick, J., Liang, Y., Sato, C., Zheng, Y., Ghani, M., Dib, S., Keith, J. *et al.* (2013) Hypermethylation of the CpG Island near the G4C2 repeat in ALS with a C9orf72 expansion. *Am. J. Hum. Genet.*, **92**, 981–989.
9. Mirkin, S.M. (2007) Expandable DNA repeats and human disease. *Nature*, **447**, 932–940.
10. Dobson-Stone, C., Hallupp, M., Loy, C.T., Thompson, E.M., Haan, E., Sue, C.M., Panegyres, P.K., Razquin, C., Seijo-Martínez, M., Rene, R. *et al.* (2013) C9ORF72 repeat expansion in Australian and Spanish frontotemporal dementia patients. *PLoS ONE*, **8**, e56899.

11. Van Langenhove, T., van der Zee, J. and Van Broeckhoven, C. (2012) The molecular basis of the frontotemporal lobar degeneration-amyotrophic lateral sclerosis spectrum. *Ann. Med.*, **44**, 817–828.
12. Brooks, B.R., Miller, R.G., Swash, M. and Munsat, T.L.; World Federation of Neurology Research Group on Motor Neuron Diseases. (2000) El Escorial revisited: revised criteria for the diagnosis of amyotrophic lateral sclerosis. *Amyotroph. Lateral Scler. Other Motor Neuron Disord.*, **1**, 293–299.
13. Rascovsky, K., Hodges, J.R., Knopman, D., Mendez, M.F., Kramer, J.H., Neuhaus, J., van Swieten, J.C., Seelaar, H., Dopper, E.G., Onyike, C.U. *et al.* (2011) Sensitivity of revised diagnostic criteria for the behavioural variant of frontotemporal dementia. *Brain*, **134**, 2456–2477.
14. Leyton, C.E., Villemagne, V.L., Savage, S., Pike, K.E., Ballard, K.J., Piguet, O., Burrell, J.R., Rowe, C.C. and Hodges, J.R. (2011) Subtypes of progressive aphasia: application of the international Consensus Criteria and validation using beta-amyloid imaging. *Brain*, **134**, 3030–3043.
15. García-Redondo, A., Dols-Icardo, O., Rojas-García, R., Esteban-Pérez, J., Cordero-Vázquez, P., Muñoz-Blanco, J.L., Catalina, I., González-Muñoz, M., Varona, L., Sarasola, E. *et al.* (2013) Analysis of the C9orf72 gene in patients with amyotrophic lateral sclerosis in Spain and different populations worldwide. *Hum. Mutat.*, **34**, 79–82.
16. Dols-Icardo, O., Suárez-Calvet, M., Hernández, I., Amer, G., Antón-Aguirre, S., Alcolea, D., Fortea, J., Boada, M., Tárraga, L., Blesa, R. *et al.* (2012) Expansion mutation in C9ORF72 does not influence plasma progranulin levels in frontotemporal dementia. *Neurobiol. Aging*, **33**, 1851.e17–1851.19.
17. van der Zee, J., Gijselinck, I., Dillen, L., Van Langenhove, T., Theuns, J., Engelborghs, S., Philtjens, S., Vandenbulcke, M., Sleegers, K., Sieben, A. *et al.* (2013) A pan-European study of the C9orf72 repeat associated with FTLD: geographic prevalence, genomic instability, and intermediate repeats. *Hum. Mutat.*, **34**, 363–373.
18. Gil-Navarro, S., Lladó, A., Rami, L., Castellví, M., Bosch, B., Bargalló, N., Lomeña, F., Reñé, R., Montagut, N., Antonell, A. *et al.* (2013) Neuroimaging and biochemical markers in the three variants of primary progressive aplasia. *Dement. Geriatr. Cogn. Disord.*, **35**, 106–117.

Neurobiology of Aging xxx (2013) 1–4

Contents lists available at ScienceDirect

Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging

Assessing the role of the *TREM2* p.R47H variant as a risk factor for Alzheimer's disease and frontotemporal dementia

Agustín Ruiz [a], Oriol Dols-Icardo [b,c], María J. Bullido [c,d,e], Pau Pastor [c,f,g], Eloy Rodríguez-Rodríguez [c,h], Adolfo López de Munain [c,i], Marian M. de Pancorbo [j], Jordi Pérez-Tur [c,k], Victoria Álvarez [l], Anna Antonell [m], Jesús López-Arrieta [e,n], Isabel Hernández [a], Lluís Tárraga [a], Mercè Boada [a,o], Alberto Lleó [b,c], Rafael Blesa [b,c], Ana Frank-García [c,e,p], Isabel Sastre [c,d,e], Cristina Razquin [c,f], Sara Ortega-Cubero [c,f,g], Elena Lorenzo [c,f], Pascual Sánchez-Juan [c,h], Onofre Combarros [c,h], Fermín Moreno [c,i], Ana Gorostidi [c,i], Xabier Elcoroaristizabal [j], Miquel Baquero [q], Eliecer Coto [l], Raquel Sánchez-Valle [m], Jordi Clarimón [b,c,*], on behalf of The dementia genetic Spanish consortium (DEGESCO)

[a] *Alzheimer Research Center and Memory Clinic, Fundació ACE, Institut Català de Neurociències Aplicades, Barcelona, Spain*
[b] *Memory Unit, Neurology Department, Hospital de Sant Pau (Sant Pau Biomedical Research Institute), Universitat Autònoma de Barcelona, Barcelona, Spain*
[c] *CIBERNED, Center for Networked Biomedical Research into Neurodegenerative Diseases, Madrid, Spain*
[d] *Centro de Biología Molecular Severo Ochoa (CSIC-UAM), Madrid, Spain*
[e] *Institute of Sanitary Research "Hospital la Paz" (IdIPaz), Madrid, Spain*
[f] *Neurogenetics Laboratory, Division of Neurosciences, Center for Applied Medical Research, University of Navarra, Pamplona, Spain*
[g] *Department of Neurology, Clínica Universidad de Navarra, University of Navarra, School of Medicine, Pamplona, Spain*
[h] *Neurology Service, University Hospital Marqués de Valdecilla (University of Cantabria and IFIMAV), Santander, Spain*
[i] *Neuroscience Area, Institute Biodonostia, and Department of Neurosciences, University of Basque Contry EHU-UPV, San Sebastián, Spain*
[j] *BIOMICs Research Group, Centro de Investigación "Lascaray" Ikergunea, Universidad del País Vasco UPV/EHU, Vitoria-Gasteiz, Spain*
[k] *Institut de Biomedicina de Valencia–CSIC, València, Spain*
[l] *Molecular Genetics Laboratory, Genetics Unit, Hospital Universitario Central de Asturias, Oviedo, Spain*
[m] *Alzheimer's disease and other cognitive disorders, Neurology Department, Hospital Clínic, IDIBAPS, Barcelona, Spain*
[n] *Memory Unit, University Hospital La Paz-Cantoblanco, Madrid, Spain*
[o] *Hospital Universitari Vall d'Hebron, Institut de Recerca, Universitat Autònoma de Barcelona (VHIR-UAB), Barcelona, Spain*
[p] *Neurology Service, Hospital Universitario La Paz (UAM), Madrid, Spain*
[q] *Neurology Service, Hospital Universitari i Politècnic La Fe, València, Spain*

ARTICLE INFO

Article history:
Received 8 May 2013
Received in revised form 5 August 2013
Accepted 10 August 2013

Keywords:
Alzheimer's disease
Frontotemporal dementia
TREM2
Genetic association
p.R47H
Rare variant

ABSTRACT

A non-synonymous genetic rare variant, rs75932628-T (p.R47H), in the *TREM2* gene has recently been reported to be a strong genetic risk factor for Alzheimer's disease (AD). Also, rare recessive mutations have been associated with frontotemporal dementia (FTD). We aimed to investigate the role of p.R47H variant in AD and FTD through a multi-center study comprising 3172 AD and 682 FTD patients and 2169 healthy controls from Spain. We found that 0.6% of AD patients carried this variant compared to 0.1% of controls (odds ratio [OR] = 4.12, 95% confidence interval [CI] = 1.21–14.00, $p = 0.014$). A meta-analysis comprising 32,598 subjects from 4 previous studies demonstrated the large effect of the p.R47H variant in AD risk (OR = 4.11, 95% CI = 2.99–5.68, $p = 5.27\times10^{-18}$). We did not find an association between p.R47H and age of onset of AD or family history of dementia. Finally, none of the FTD patients harbored this genetic variant. These data strongly support the important role of p.R47H in AD risk, and suggest that this rare genetic variant is not related to FTD.

1. Introduction

The rapid population growth in recent centuries and the evolutionary forces that shape allelic variation over time have led to a large number of rare genetic variants in the human genome, and they seem to vastly exceed the number of common alleles

* Corresponding author at: Genetics of Neurodegenerative Disorders Unit, IIB-Sant Pau, Neurology Department, Hospital de la Santa Creu i Sant Pau, Sant Antoni Mª Claret 167, 08025 Barcelona, Spain. Tel.: +34 932919050 ext.8230; fax: +34 935565602.
E-mail address: jclarimon@santpau.cat (J. Clarimón).

0197-4580/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.neurobiolaging.2013.08.011

(Tennessen et al., 2012). A substantial fraction of these rare variants may have functional consequences and therefore can contribute to the allelic architecture of complex diseases (Pritchard, 2001). A very recent example of a rare genetic variant with large individual effect is the non-synonymous amino acid substitution p.R47H (rs75932628) in the gene encoding the triggering receptor expressed on myeloid cells 2 (*TREM2*) and its association with the risk for sporadic Alzheimer's disease (AD) (Guerreiro et al., 2013a; Jonsson et al., 2013). This association has also been replicated in a cohort of French patients with an early-onset form of AD (age of onset ≤65 years) (Pottier et al., 2013), and in a Spanish study with a series of patients that comprised AD patients with both late-onset and early-onset disease (Benitez et al., 2013). Interestingly, homozygous mutations in *TREM2* have been described in patients with an unusual early-onset frontotemporal dementia (FTD) from consanguineous families living in the Anatolian region of Turkey (Guerreiro et al., 2013b) and in a Colombian family (Giraldo et al., 2013), thus broadening the clinical heterogeneity spectrum caused by mutations in this particular locus. In addition, homozygous mutations in *TREM2* are also the cause of Nasu-Hakola disease, which is mainly characterized by pre-senile FTD and cystic bone lesions (Paloneva et al., 2002).

In the present work, we aimed to evaluate how this non-synonymous amino acid change contributes to the risk of early- and late-onset forms of AD or FTD by studying a large cohort of patients and controls from Spanish origin.

2. Methods

2.1. Study subjects

A total of 3,172 AD patients (mean age at onset 74.8 ± 9.9 years, 68.5% women), 682 FTD patients (mean age at onset 64.8 ± 9.9 years, 46.8% women), and 2169 healthy controls (mean age at clinical assessment 75.4 ± 10.3 years, 58.2% women) were collected through a collaborative effort involving 11 specialized centers across the country. All individuals were Spanish and of European origin. Among AD patients, 466 were classified as having early-onset AD (EOAD; age of onset ≤65 years). Patients were diagnosed using established clinical criteria for AD (McKhann et al., 1984) or FTD (Neary et al., 1998). All participants or their families provided written informed consent, and the study was approved by the respective ethics committees.

2.2. Genotyping

Genotyping of the rs75932628 (p.R47H) variant was performed using 3 approaches: TaqMan SNP Genotyping Assays (Applied Biosystems, Foster City, CA), High Resolution Melting (Eco-PCR, Illumina, San Diego, CA), and KASPar (KBioscience, Berlin, Germany). A human DNA sample carrying a T-allele (rs75932628-T) in a heterozygous state was distributed to all genotyping centers and was included as a positive control in all genotyping plates. Given that 3 different genotyping methods were used and different genotyping centers contributed data for this study, the concordance rate was analyzed for the distributed control and internal blanks included on each plate. Specifically, the same positive (heterozygous) DNA was included in thirty 48-well Eco PCR plates (Illumina) and twelve 384-well plates (for KASPar and Taqman technologies). Perfect concordance between observed and expected results was obtained for all positive and blank controls (80/80 experiments; 100%).

2.3. Statistical analysis

Genotype frequency comparisons were performed by the Fisher exact test. Differences between groups were analyzed by the Student *t* test for continuous data. Multiple logistic regression models were used to adjust for covariates such as age, sex, and *APOE*-ε4 status (presence/absence). Unadjusted and adjusted (sex and APOE-ε4 status in a dominant model) linear regression models were used to explore the effect of p.R47H variant in the age at onset of AD. Data were analyzed using the Statistical Package for the Social Sciences, version 19.0.0 (SPSS Inc, Chicago, IL). Genetic interactions (epistasis) were assessed by the synergy factor analysis (Cortina-Borja et al., 2009). Meta-analysis was conducted using the inverse variance method (fixed-effects model) in Episheet Excel application according to Fleiss (Fleiss, 1993). Fixed- and random-effects meta-analyses were automatically generated by Episheet. The weighting of each study was calculated using the estimated standard errors. The final meta-analysis results and forest plot for TREM2 p.R47H showing association results in all available data were derived using OpenMeta (Wallace et al., 2009). Power calculations were performed with PS software (version 2.1.30).

3. Results

A total of 6023 individuals were genotyped for the p.R47H polymorphism, and distribution of genotype frequencies across phenotypes is presented in Table 1. The T allele at the rs75932628 biallelic polymorphism was carried by 0.6% of AD patients and 0.1% of controls ($p = 0.014$), and yielded an odds ratio (OR) of 4.12 (95% confidence interval [CI] = 1.21–14.00), almost identical to the risk conferred by the *APOE*-ε4 allele (4.2, Table 1). Although not statistically significant, age at onset of AD was slightly less in rs75932628-T carriers than in non-carriers (70 ± 6.0 and 74.8 ± 9.9 years, respectively, $p = 0.08$). To effectively detect whether the rs75932628-T variant has a role in the age at onset of AD, linear regression–based analyses were conducted. Unadjusted linear regression revealed a trend toward association between age at onset of AD and *TREM2* p.R47H carrier status (B = −4.41; 95% CI = −9.28 to 0.47; $p = 0.078$). Sex and *APOE*-ε4 (dominant model) adjusted linear regression analysis yielded similar results (B = −3.87; 95% CI = −8.55 to 0.81; $p = 0.11$). Data regarding family history of dementia were available for 14 of 18 AD patients carrying the rs75932628-T allele. Of these patients, only 5 (35.7%) reported a positive family history of dementia. This percentage was almost identical to the prevalence of family history of dementia in rs75932628-T noncarriers (35.3%, $p = 0.974$). To further address the association between the p.R47H variant and the risk of AD, and to compare the observed effect size of p.R47H among different populations, we conducted a meta-analysis using our data and the available data from the literature (Benitez et al., 2013; Guerreiro et al., 2013a, 2013b; Jonsson et al., 2013; Pottier et al., 2013). In total, we analyzed the genotypes of 32,598 subjects comprising 12,967 patients and 19,631 controls. The summary OR under a fixed-effect model showed that individuals with the p.R47H variant were 4.11 times more likely to develop AD than noncarriers (95%

Table 1
Frequency of *TREM2* p.R47H and *APOE*-ε4 carriers across phenotypes

	TREM2 p.R47H n (frequency %)	*APOE*-ε4 n (frequency %)
Controls	3 (0.14)	257 (16.1)
AD patients	18 (0.57)	1,264 (44.8)
FTD patients	—	64 (24.5)
OR [95% CI] (AD vs. C)	4.12 [1.21–14.00], $p = 0.014$	4.23 [3.61–4.93], $p = 6.9\times10^{-83}$
OR [95% CI] (FTD vs. C)	—, $p = 1$	1.69 [1.24–2.31], $p = 0.001$

Key: AD, Alzheimer's disease; CI, confidence interval; FTD, frontotemporal dementia; OR, odds ratio.

CI = 2.99–5.68, $p = 5.27 \times 10^{-18}$, Fig. 1). No evidence of heterogeneity between studies was detected (Q = 2.17, $p > 0.98$ with 9 df).

We next assessed the effect of the *APOE*-ε4 allele on the association between rs75932628-T and the risk of AD. The risk of the p.R47H variant was no longer significant after adjustment for the *APOE*-ε4 allele ($p = 0.34$). Since there were significant differences between the frequency of rs75932628-T in carriers of the *APOE*-ε4 allele compared to noncarriers (1.1% and 0.3%, respectively, $p = 0.007$), we then evaluated whether the *TREM2* association was indeed confounded by the presence of *APOE*-ε4 in rs75932628-T carriers through a meta-analysis that included 2 previous studies with 6 populations (Jonsson et al., 2013; Pottier et al., 2013). This analysis showed no difference in the frequency of rs75932628-T carriers according to *APOE*-ε4 status ($p = 0.28$, Supplementary. Fig. 1). No significant interaction between *APOE*-ε4 and rs75932628-T resulted from the logistic regression model ($p = 0.577$). Evaluation of this interaction by the synergy factor (SF) analysis, which measures both the size and significance of genetic interactions (Cortina-Borja et al., 2009), corroborated the lack of synergy between *APOE*-ε4 and rs75932628-T (SF = 1.31, $p = 0.43$).

The study of the p.R47H variant in the FTD group (n = 682) did not disclose any carrier of this genetic variant.

4. Discussion

The role of rare genetic variants in common disorders has been fueled in the last few years by the appearance of next-generation sequencing technologies. Two independent works have recently demonstrated the power of these techniques. After a large-scale analysis of sequencing data, they identified the rare p.R47H substitution as a major genetic risk factor for AD (Guerreiro et al., 2013a; Jonsson et al., 2013). In the present work, we were able to replicate this finding by analyzing a large case-control cohort comprising more than 6000 individuals. The effect size of the *TREM2* rs75932628-T rare variant was consistent with those reported previously (Benitez et al., 2013; Guerreiro et al., 2013a; Jonsson et al., 2013), and reached the same magnitude as the *APOE*-ε4 allele, the most important genetic risk factor for AD. Our meta-analysis comprising >32,000 subjects strongly supports the important role of the p.R47H non-synonymous variant in AD risk.

We were not able to replicate the recently reported association of rs75932628-T and EOAD risk in a French population (Pottier et al., 2013), despite the frequency of EOAD patients carrying the p.R47H variant being slightly higher than that in controls (0.4% and 0.1%, respectively). A possible explanation for this lack of replication is that the frequency of the rs75932628-T variant in the Spanish population is much lower than that in the French population (0.14% and 0.5%, respectively, in controls, and 0.4% and 2.0% in patients). Therefore, even though our sample size was almost double that of Pottier et al., the much lower frequency of the rs75932628-T in our population could have led to an insufficient statistical power. The present study had a power of 46% ($\alpha = 0.05$) to detect a risk equal to that reported in the French analysis. It is important to note, however, that patients carrying the rs75932628-T variant had a slightly, although not significantly, earlier age of onset, thus raising the question as to whether this genetic variant could modulate the age of onset of AD.

We did not find any interaction between the ε4 allele of the *APOE* gene and the p.R47H variant. Nevertheless, the *APOE*-ε4 adjusted risk of the rs75932628-T variant resulted in a loss of statistical significance. The high prevalence of the APOE-E4 isoform in AD patients, in conjunction with its prominent effect on AD risk, may explain this somewhat unexpected outcome. However, our meta-analysis to evaluate the effect of *APOE*-ε4 on the association of rs75932628-T with AD strongly indicates the independence of both genes in the risk of AD. Therefore, co-occurrence of both alleles (rs75932628-T and *APOE*-ε4) in our AD series was not confirmed in other populations and might be due to random effects.

Our results suggest that the p.R47H variant does not contribute to the risk of common forms of FTD in the Spanish population. This outcome does not agree with recent data that included 609 North American FTD patients (Rayaprolu et al., 2013). In that case, the association was found within the clinical series but not among the pathologically confirmed cases. One possible explanation for this discrepancy could be confounder factors that are inherent to clinical diagnosis in neurodegenerative dementias. Another possible reason could be related to population stratification in the North American series. Because rare genetic variants are more prone to geographic stratification, studies that do not include a very homogenous population might be more prone to false-positive outcomes (Tennessen et al., 2012). Finally, discrepancies between our data and those of Rayaprolu et al. could pertain to population-specific genetic causes related to complex diseases such as FTD. Therefore, population-specific catalogs of variants, as well as careful collection of information about ancestry, will be mandatory to evaluate the role of rare variants within *TREM2* gene in neurodegenerative disorders.

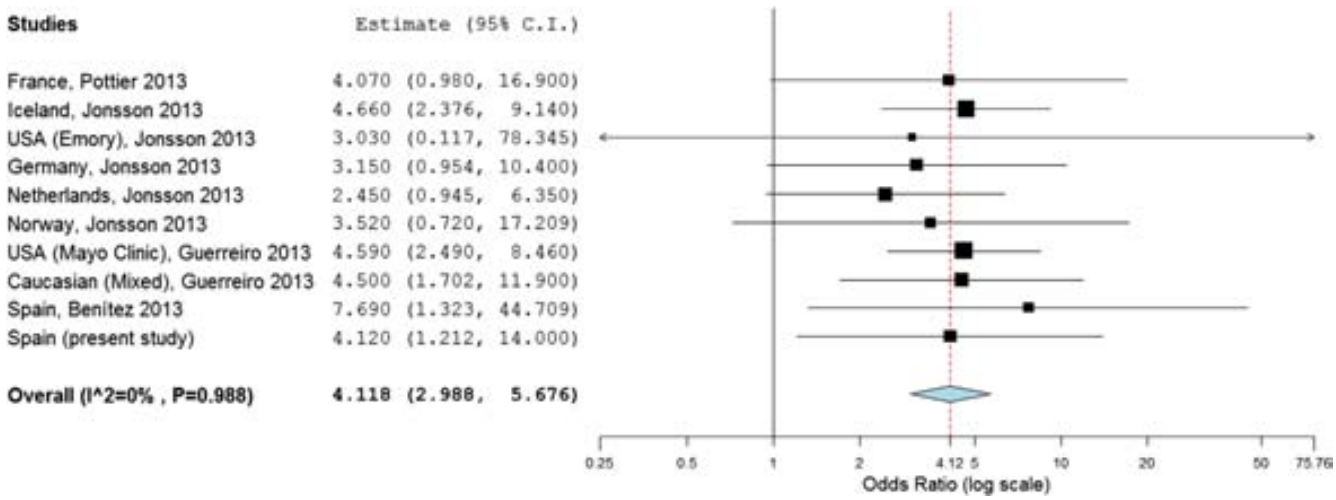


Fig. 1. Forest plot with the rs7593268-T allelic odds ratios from published studies and overall odds ratio.

Disclosure statement

The authors declare no actual or potential conflicts of interest.

Acknowledgements

We would thank the patients, their families, and the control subjects for their participation in this study. Thes study was supported by grants from Instituto de Salud Carlos III (PI12/01311 and 12/00013) and grants from the Ministry of Science (SAF2010-15558, SAF2009-10434), Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED, Spain), Consolider (CSD2010-00045), Ayudas a proyectos de investigación sanitaria (Departamento de Sanidad y Consumo del Gobierno Vasco, Ref. 2009111083), and the Department of Health of the Government of Navarra (refs. 13085 and 3/2008). C.R. held, during the period 2009–2013, a "Torres Quevedo" fellowship from the Spanish Ministry of Science and Technology, co-financed by the European Social Fund. Fundació ACE researchers are indebted to Trinitat Port-Carbó and her family who are supporting Fundació ACE scientific programs. We also would like to thank Drs P. Gil and P. Coria for their cooperation in the generation of the case-control samples, and Drs José Luis Molinuevo, Albert Lladó, and Lorena Rami (from Hospital Clínic), Drs Juan Fortea and Daniel Alcolea (from Hospital Sant Pau), Dr J.A. Burguera (from Hospital Universitari i Politècnic La Fe), and Dr Antonio Salazar (from Direcció General de Salut Pública de la Generalitat Valenciana) for their effort in clinical evaluation and sample recruitment, and Carolyn Newey for editorial assistance.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.neurobiolaging.2013.08.011.

References

Benitez, B.A., Cooper, B., Pastor, P., Jin, S.C., Lorenzo, E., Cervantes, S., Cruchaga, C., 2013. TREM2 is associated with the risk of Alzheimer's disease in Spanish population. Neurobiol. Aging 34, 1711 e15–1711 e17.

Cortina-Borja, M., Smith, A.D., Combarros, O., Lehmann, D.J., 2009. The synergy factor: a statistic to measure interactions in complex diseases. BMC Res. Notes 2, 105.

Fleiss, J.L., 1993. The statistical basis of meta-analysis. Stat. Methods Med. Res. 2, 121–145.

Giraldo, M., Lopera, F., Siniard, A.L., Corneveaux, J.J., Schrauwen, I., Carvajal, J., Muñoz, C., Ramírez-Restrepo, M., Gaiteri, C., Myers, A.J., Caselli, R.J., Kosik, K.S., Reiman, E.M., Huentelman, M.J., 2013. Variants in triggering receptor expressed on myeloid cells 2 are associated with both behavioral variant frontotemporal lobar degeneration and Alzheimer's disease. Neurbiol. Aging 34, 2077.e11–8.

Guerreiro, R., Wojtas, A., Bras, J., Carrasquillo, M., Rogaeva, E., Majounie, E., Cruchaga, C., Sassi, C., Kauwe, J.S., Younkin, S., Hazrati, L., Collinge, J., Pocock, J., Lashley, T., Williams, J., Lambert, J.C., Amouyel, P., Goate, A., Rademakers, R., Morgan, K., Powell, J., St George-Hyslop, P., Singleton, A., Hardy, J., 2013a. TREM2 variants in Alzheimer's disease. N. Engl. J. Med. 368, 117–127.

Guerreiro, R.J., Lohmann, E., Bras, J.M., Gibbs, J.R., Rohrer, J.D., Gurunlian, N., Dursun, B., Bilgic, B., Hanagasi, H., Gurvit, H., Emre, M., Singleton, A., Hardy, J., 2013b. Using exome sequencing to reveal mutations in TREM2 presenting as a frontotemporal dementia-like syndrome without bone involvement. JAMA Neurol. 70, 78–84.

Jonsson, T., Stefansson, H., Steinberg, S., Jonsdottir, I., Jonsson, P.V., Snaedal, J., Bjornsson, S., Huttenlocher, J., Levey, A.I., Lah, J.J., Rujescu, D., Hampel, H., Giegling, I., Andreassen, O.A., Engedal, K., Ulstein, I., Djurovic, S., Ibrahim-Verbaas, C., Hofman, A., Ikram, M.A., van Duijn, C.M., Thorsteinsdottir, U., Kong, A., Stefansson, K., 2013. Variant of TREM2 associated with the risk of Alzheimer's disease. N. Engl. J. Med. 368, 107–116.

McKhann, G., Drachman, D., Folstein, M., Katzman, R., Price, D., Stadlan, E.M., 1984. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 34, 939–944.

Neary, D., Snowden, J.S., Gustafson, L., Passant, U., Stuss, D., Black, S., Freedman, M., Kertesz, A., Robert, P.H., Albert, M., Boone, K., Miller, B.L., Cummings, J., Benson, D.F., 1998. Frontotemporal lobar degeneration: a consensus on clinical diagnostic criteria. Neurology 51, 1546–1554.

Paloneva, J., Manninen, T., Christman, G., Hovanes, K., Mandelin, J., Adolfsson, R., Bianchin, M., Bird, T., Miranda, R., Salmaggi, A., Tranebjaerg, L., Konttinen, Y., Peltonen, L., 2002. Mutations in two genes encoding different subunits of a receptor signaling complex result in an identical disease phenotype. Am. J. Hum. Genet. 71, 656–662.

Pottier, C., Wallon, D., Rousseau, S., Rovelet-Lecrux, A., Richard, A.C., Rollin-Sillaire, A., Frebourg, T., Campion, D., Hannequin, D., 2013. TREM2 R47H Variant as a Risk Factor for Early-Onset Alzheimer's Disease. J. Alzheimers Dis. 35, 45–49.

Pritchard, J.K., 2001. Are rare variants responsible for susceptibility to complex diseases? Am. J. Hum. Genet. 69, 124–137.

Rayaprolu, S., Mullen, B., Baker, M., Lynch, T., Finger, E., Seeley, W.W., Hatanpaa, K.J., Lomen-Hoerth, C., Kertesz, A., Bigio, E.H., Lippa, C., Josephs, K.A., Knopman, D.S., White 3rd, C.L., Caselli, R., Mackenzie, I.R., Miller, B.L., Boczarska-Jedynak, M., Opala, G., Krygowska-Wajs, A., Barcikowska, M., Younkin, S.G., Petersen, R.C., Ertekin-Taner, N., Uitti, R.J., Meschia, J.F., Boylan, K.B., Boeve, B.F., Graff-Radford, N.R., Wszolek, Z.K., Dickson, D.W., Rademakers, R., Ross, O.A., 2013. TREM2 in neurodegeneration: evidence for association of the p.R47H variant with frontotemporal dementia and Parkinson's disease. Mol. Neurodegener. 8, 19.

Tennessen, J.A., Bigham, A.W., O'Connor, T.D., Fu, W., Kenny, E.E., Gravel, S., McGee, S., Do, R., Liu, X., Jun, G., Kang, H.M., Jordan, D., Leal, S.M., Gabriel, S., Rieder, M.J., Abecasis, G., Altshuler, D., Nickerson, D.A., Boerwinkle, E., Sunyaev, S., Bustamante, C.D., Bamshad, M.J., Akey, J.M., 2012. Evolution and functional impact of rare coding variation from deep sequencing of human exomes. Science 337, 64–69.

Wallace, B.C., Schmid, C.H., Lau, J., Trikalinos, T.A., 2009. Meta-Analyst: software for meta-analysis of binary, continuous and diagnostic data. BMC Med. Res. Methodol. 9, 80.

Frontotemporal dementia and its subtypes: a genome-wide association study

Raffaele Ferrari, Dena G Hernandez*, Michael A Nalls*, Jonathan D Rohrer*, Adaikalavan Ramasamy, John B J Kwok, Carol Dobson-Stone, William S Brooks, Peter R Schofield, Glenda M Halliday, John R Hodges, Olivier Piguet, Lauren Bartley, Elizabeth Thompson, Eric Haan, Isabel Hernández, Agustín Ruiz, Mercè Boada, Barbara Borroni, Alessandro Padovani, Carlos Cruchaga, Nigel J Cairns, Luisa Benussi, Giuliano Binetti, Roberta Ghidoni, Gianluigi Forloni, Daniela Galimberti, Chiara Fenoglio, Maria Serpente, Elio Scarpini, Jordi Clarimón, Alberto Lleó, Rafael Blesa, Maria Landqvist Waldö, Karin Nilsson, Christer Nilsson, Ian R A Mackenzie, Ging-Yuek R Hsiung, David M A Mann, Jordan Grafman, Christopher M Morris, Johannes Attems, Timothy D Griffiths, Ian G McKeith, Alan J Thomas, P Pietrini, Edward D Huey, Eric M Wassermann, Atik Baborie, Evelyn Jaros, Michael C Tierney, Pau Pastor, Cristina Razquin, Sara Ortega-Cubero, Elena Alonso, Robert Perneczky, Janine Diehl-Schmid, Panagiotis Alexopoulos, Alexander Kurz, Innocenzo Rainero, Elisa Rubino, Lorenzo Pinessi, Ekaterina Rogaeva, Peter St George-Hyslop, Giacomina Rossi, Fabrizio Tagliavini, Giorgio Giaccone, James B Rowe, Johannes C M Schlachetzki, James Uphill, John Collinge, Simon Mead, Adrian Danek, Vivianna M Van Deerlin, Murray Grossman, John Q Trojanowski, Julie van der Zee, William Deschamps, Tim Van Langenhove, Marc Cruts, Christine Van Broeckhoven, Stefano F Cappa, Isabelle Le Ber, Didier Hannequin, Véronique Golfier, Martine Vercelletto, Alexis Brice, Benedetta Nacmias, Sandro Sorbi, Silvia Bagnoli, Irene Piaceri, Jørgen E Nielsen, Lena E Hjermind, Matthias Riemenschneider, Manuel Mayhaus, Bernd Ibach, Gilles Gasparoni, Sabrina Pichler, Wei Gu, Martin N Rossor, Nick C Fox, Jason D Warren, Maria Grazia Spillantini, Huw R Morris, Patrizia Rizzu, Peter Heutink, Julie S Snowden, Sara Rollinson, Anna Richardson, Alexander Gerhard, Amalia C Bruni, Raffaele Maletta, Francesca Frangipane, Chiara Cupidi, Livia Bernardi, Maria Anfossi, Maura Gallo, Maria Elena Conidi, Nicoletta Smirne, Rosa Rademakers, Matt Baker, Dennis W Dickson, Neill R Graff-Radford, Ronald C Petersen, David Knopman, Keith A Josephs, Bradley F Boeve, Joseph E Parisi, William W Seeley, Bruce L Miller, Anna M Karydas, Howard Rosen, John C van Swieten, Elise G P Dopper, Harro Seelaar, Yolande A L Pijnenburg, Philip Scheltens, Giancarlo Logroscino, Rosa Capozzo, Valeria Novelli, Annibale A Puca, Massimo Franceschi, Alfredo Postiglione, Graziella Milan, Paolo Sorrentino, Mark Kristiansen, Huei-Hsin Chiang, Caroline Graff, Florence Pasquier, Adeline Rollin, Vincent Deramecourt, Florence Lebert, Dimitrios Kapogiannis, Luigi Ferrucci, Stuart Pickering-Brown, Andrew B Singleton†, John Hardy†, Parastoo Momeni†*

Lancet Neurol 2014; 13: 686–99

This online publication has been corrected. The corrected version first appeared at thelancet.com/neurology on June 17, 2014

See Comment page 643

*Contributed equally

†Joint last authors

Laboratory of Neurogenetics, Department of Internal Medicine, Texas Tech University Health Science Center, Lubbock, Texas, USA (R Ferrari PhD, P Momeni PhD); Reta Lila Weston Research Laboratories, Department of Molecular Neuroscience, UCL Institute of Neurology, London, UK (R Ferrari, D G Hernandez MSc, J D Rohrer PhD, A Ramasamy PhD, Prof J Hardy PhD); Laboratory of Neurogenetics, National Institute on Aging, National Institutes of Health, Bethesda, MD, USA (D G Hernandez, M A Nalls PhD, A B Singleton PhD); Clinical Research Branch, National Institute on Aging, Baltimore, MD, USA (L Ferrucci MD); Institute of Brain, Behaviour and Mental Health, Faculty of Medical and Human Sciences, University of Manchester, Manchester, UK

Summary

Background Frontotemporal dementia (FTD) is a complex disorder characterised by a broad range of clinical manifestations, differential pathological signatures, and genetic variability. Mutations in three genes—*MAPT*, *GRN*, and *C9orf72*—have been associated with FTD. We sought to identify novel genetic risk loci associated with the disorder.

Methods We did a two-stage genome-wide association study on clinical FTD, analysing samples from 3526 patients with FTD and 9402 healthy controls. To reduce genetic heterogeneity, all participants were of European ancestry. In the discovery phase (samples from 2154 patients with FTD and 4308 controls), we did separate association analyses for each FTD subtype (behavioural variant FTD, semantic dementia, progressive non-fluent aphasia, and FTD overlapping with motor neuron disease [FTD-MND]), followed by a meta-analysis of the entire dataset. We carried forward replication of the novel suggestive loci in an independent sample series (samples from 1372 patients and 5094 controls) and then did joint phase and brain expression and methylation quantitative trait loci analyses for the associated ($p<5\times10^{-8}$) single-nucleotide polymorphisms.

Findings We identified novel associations exceeding the genome-wide significance threshold ($p<5\times10^{-8}$). Combined (joint) analyses of discovery and replication phases showed genome-wide significant association at 6p21.3, *HLA* locus (immune system), for rs9268877 ($p=1\cdot05\times10^{-8}$; odds ratio=1·204 [95% CI 1·11–1·30]), rs9268856 ($p=5\cdot51\times10^{-9}$; 0·809 [0·76–0·86]) and rs1980493 (p value=$1\cdot57\times10^{-8}$, 0·775 [0·69–0·86]) in the entire cohort. We also identified a potential novel locus at 11q14, encompassing *RAB38*/*CTSC* (the transcripts of which are related to lysosomal biology), for the behavioural FTD subtype for which joint analyses showed suggestive association for rs302668 ($p=2\cdot44\times10^{-7}$; 0·814 [0·71–0·92]). Analysis of expression and methylation quantitative trait loci data suggested that these loci might affect expression and methylation in *cis*.

Interpretation Our findings suggest that immune system processes (link to 6p21.3) and possibly lysosomal and autophagy pathways (link to 11q14) are potentially involved in FTD. Our findings need to be replicated to better define the association of the newly identified loci with disease and to shed light on the pathomechanisms contributing to FTD.

Funding The National Institute of Neurological Disorders and Stroke and National Institute on Aging, the Wellcome/MRC Centre on Parkinson's disease, Alzheimer's Research UK, and Texas Tech University Health Sciences Center.

Introduction

Frontotemporal dementia (FTD) is the second most common form of young-onset dementia after Alzheimer's disease and comprises about 10–20% of all dementias worldwide.[1] FTD occurs in about three to 15 per 100 000 individuals aged between 55 years and 65 years.[2] The disease has a slow and subtle onset: it is familial in 30–50% of patients and affects men and women almost equally.[3] The main clinical syndromes are the behavioural variant[1,4] and the language variants (semantic dementia and progressive nonfluent aphasia).[1,5] FTD can also co-occur with motor neuron disease (FTD-MND), and atypical parkinsonian disorders.[3] The molecular pathology is heterogeneous and based on the type of neuronal lesions and protein inclusions: 40% or more of patients have frontotemporal lobar degeneration (FTLD) with tau pathology (FTLD-tau), about 50% have TDP-43 (TAR DNA-binding protein 43) pathology (FTLD-TDP),[6] and the remaining 10% have inclusions positive for fused in sarcoma (FUS; FTLD-FUS) or ubiquitin/p62 (FTLD-UPS [ubiquitin proteasome system]).[7] Mutations in three main genes are commonly associated with FTD: the microtubule-associated protein tau (*MAPT*),[8] granulin (*GRN*),[9,10] and *C9orf72*.[11–15] Mutations in the charged multivesicular body protein 2B (*CHMP2B*), the valosin-containing protein (*VCP*), and ubiquilin 2 (*UBQLN2*) genes are rare causes of disease.[13,16] Findings from a previous genome-wide association study (GWAS) of neuropathologically confirmed FTLD-TDP (515 patients *vs* 2509 controls) showed *TMEM106B* to be a disease risk factor.[17]

We did a larger GWAS in samples from people with clinical FTD, and we report results for the discovery, replication, and joint-phase analyses, as well as for assessment of the effect on expression and methylation quantitative trait loci (QTL) exerted by associated or suggestive SNPs. We aimed to identify novel genetic risk loci associated with FTD and its subtypes.

Methods

Study population

44 international research groups (appendix) contributed samples to this two-stage (discovery phase and replication phase) GWAS of clinical FTD. The patients included in the discovery phase were diagnosed according to the Neary criteria[1] for FTD, whereas those included in the replication phase were diagnosed according to the Neary criteria,[1] or the revised criteria for behavioural FTD[4] and the language variants of FTD[5] at every collaborative site. For each patient, the diagnosis was made by a neurologist with an interest in FTD or, in a minority (70 [3%] of 2621 patients), by pathological diagnosis. To cover the most relevant FTD clinical signatures, we included patients diagnosed with behavioural FTD, semantic dementia, progressive nonfluent aphasia, or FTD-MND.[18] We reviewed all patients with a diagnosis of language impairment to exclude cases of the logopenic variant of primary progressive aphasia,[5] most of which are associated with Alzheimer's disease pathology. Samples were obtained from Australia, Belgium, Denmark, France, Germany, Italy, the Netherlands, North America (USA and Canada), Spain, Sweden, and the UK and all patients were of confirmed European ancestry (to reduce genetic heterogeneity).

DNA was collected at the three institutions leading this project: the Department of Molecular Neuroscience at University College London (UCL), UK; the Laboratory of Neurogenetics of the National Institute on Aging at the National Institutes of Health (NIH), MD, USA; and the Laboratory of Neurogenetics at the Texas Tech University Health Sciences Center (TTUHSC), TX, USA. All samples were anonymous and stored with a patient-specific coded identification number. Each DNA sample was assessed for quality with gel electrophoresis and DNA concentrations were assessed via spectrophotometer (Nanodrop; Wilmington, DE, USA) or fluorometer (Qubit; Life Technologies, Grand Island, NY, USA). Samples from non-overlapping patients were genotyped at the Laboratory of Neurogenetics of the National Institute on Aging, NIH (40%), or at the core facility at the Institute of Child Health, UCL (60%). We obtained standardised clinical, pathological, and genetic data for each patient from all the collaborating groups (appendix). Sporadic cases along with probands from FTD families were included in the study. We excluded carriers of mutations in *MAPT* and *GRN*. We did not exclude individuals with *C9orf72* expansions because this locus was identified subsequent to sample collection. After quality control of genotyping data and detailed assessment of the clinical diagnosis, we used 2154 and 1372 samples in the discovery phase and replication phase, respectively, for association analysis (table 1). In total, after quality control, we analysed 3526 FTD samples (table 1). Further details about cases included in the study are provided in the appendix.

Control samples for the discovery phase were taken from studies previously done at the Laboratory of Neurogenetics of the National Institute on Aging at the NIH or at UCL. Control individuals were matched to patients on the basis of population ancestry and genotyping platform. Aggregate data for control samples were merged based on overlapping single-nucleotide polymorphisms (SNPs). The selected 7444 control samples were from France, Germany, Italy, the Netherlands, Sweden, the UK, and USA, and were used as controls in previous GWAS;[19] all individuals had given consent for their samples to be used as controls. All were free of neurological illness at the time of sampling, but most had not been screened for the absence of a family history of FTD. For each patient, at least two controls were matched based on compatibility of genetic ancestry estimates by principal components analysis to accommodate the lack of precisely matched clinical controls. After quality control, we included 4308

(Prof J S Snowden PhD, S Rollinson PhD, Prof S Pickering-Brown PhD); **Neuroscience Research Australia, Sydney, NSW, Australia** (J B J Kwok PhD, C Dobson-Stone PhD, W S Brooks MBBS, Prof P R Schofield DSc, Prof G M Halliday PhD, Prof J R Hodges MD, O Piguet PhD, L Bartley MSc); **University of New South Wales, Sydney, NSW, Australia** (J B J Kwok, C Dobson-Stone, W S Brooks, Prof P R Schofield, Prof G M Halliday, Prof J R Hodges, O Piguet); **South Australian Clinical Genetics Service, SA Pathology at Women's and Children's Hospital, North Adelaide, SA, Australia** (E Thompson MD, Prof E Haan MBBS); **Department of Paediatrics, University of Adelaide, Adelaide, SA, Australia** (E Thompson, Prof E Haan); **Memory Clinic of Fundació ACE, Institut Català de Neurociències Aplicades, Barcelona, Spain** (I Hernández MD, A Ruiz MD, M Boada MD); **Hospital Universitari Vall d'Hebron–Institut de Recerca, Universitat Autonoma de Barcelona (VHIR-UAB), Barcelona, Spain** (M Boada); **Neurology Clinic, University of Brescia, Brescia, Italy** (B Borroni MD, Prof A Padovani MD); **Department of Psychiatry** (C Cruchaga PhD), **Hope Center** (C Cruchaga, Prof N J Cairns PhD), **Washington University School of Medicine, St Louis, Missouri, USA; Department of Pathology and Immunology, Washington University, St Louis, Missouri, USA** (Prof N J Cairns); **NeuroBioGen Lab—Memory Clinic, IRCCS Istituto Centro San Giovanni di Dio Fatebenefratelli, Brescia, Italy** (L Benussi PhD, G Binetti MD); **Proteomics Unit, IRCCS Istituto Centro San Giovanni di Dio Fatebenefratelli, Brescia, Italy** (R Ghidoni PhD); **Biology of Neurodegenerative Disorders, IRCCS Istituto di Ricerche Farmacologiche Mario Negri, Milano, Italy** (G Forloni PhD); **University of Milan, Milan, Italy** (D Galimberti PhD, C Fenoglio PhD, M Serpente PhD, E Scarpini MD); **Fondazione Cà Granda, IRCCS Ospedale Maggiore Policlinico, Milan, Italy** (D Galimberti, C Fenoglio, M Serpente, E Scarpini); **Memory Unit, Neurology Department**

	Samples collected (n)			Samples included in analysis (n)			Samples from women (% [n/N])		Mean age at onset (years [range]; N)	
	Discovery phase	Replication phase	Total	Discovery phase	Replication phase	Total	Discovery phase	Replication phase	Discovery phase	Replication phase
Australia	0	138	138	0	121	121	NA	36% (44/121)	NA	59 (32–77); 112
Belgium	240	51	291	191	42	233	46% (88/191)	29% (12/42)	63 (29–90); 191	64 (43–84); 42
Canada	25	37	62	24	29	53	52% (12/23)	57% (8/14)	64 (43–85); 15	59 (43–75); 9
Denmark	35	0	35	7	0	7	71% (5/7)	NA	57 (40–62); 7	NA
France	238	54	292	205	42	247	44% (91/205)	48% (20/42)	62 (39–79); 190	NA
Germany	349	34	383	320	33	353	NA	50% (15/30)	61 (36–83); 243	57 (29–72); 30
Italy	1035	563	1598	564	371	935	53% (297/561)	45% (168/371)	64 (31–83); 429	65 (31–87); 353
Netherlands	333	93	426	250	77	327	52 (129/250)	40% (31/77)	58 (29–76); 250	61 (51–69); 59
Spain	100	330	430	0	309	309	NA	43% (133/309)	NA	65 (32–89); 308
Sweden	26	112	138	18	98	116	56% (10/18)	61% (60/98)	57 (38–75); 16	62 (28–78); 93
UK	494	372	866	401	284	685	43% (171/400)	40% (108/272)	60 (23–83); 372	61 (35–86); 167
USA	706	209	915	579	175	754	44% (257/579)	49% (85/174)	60 (23–85); 520	63 (24–93); 120
Total	3581	1993	5574	2559 (2154*)	1581 (1372*)	4140 (3526*)	47% (1186/2552)	44% (684/1550)	61 (23–90); 2233	62 (24–93); 1293

NA=not applicable. *The number of the samples that passed genotyping data quality control and were used for association analyses.

Table 1: **Sample characteristics**

and Sant Pau Biomedical Research Institute, Hospital de la Santa Creu i Sant Pau, Universitat Autònoma de Barcelona, Barcelona, Spain (J Clarimón PhD, A Lleó MD, R Blesa MD)**; Center for Networker Biomedical Research in Neurodegenerative Diseases (CIBERNED), Madrid, Spain** (J Clarimón, A Lleó, R Blesa, P Pastor MD, S Ortega-Cubero MD)**; Unit of Geriatric Psychiatry** (M L Waldö MD, K Nilsson PhD)**, Clinical Memory Research Unit** (C Nilsson PhD)**, Department of Clinical Sciences, Lund University, Sweden; Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, Canada** (Prof I R A Mackenzie MD)**; Division of Neurology, University of British Columbia, Vancouver, Canada** (G-Y R Hsiung MD)**; Institute of Brain, Behaviour and Mental Health, University of Manchester, Salford Royal Hospital, Stott Lane, Salford, UK** (Prof D M A Mann PhD)**; Rehabilitation Institute of Chicago, Departments of Physical Medicine and Rehabilitation, Psychiatry, and Cognitive Neurology and Alzheimer's Disease Center; Feinberg School of Medicine, Northwestern University, IL, USA** (Prof J Grafman PhD, C M Morris PhD, Prof J Attems MD, Prof T D Griffiths FMedSci)**; Department of Psychology,**

control samples in this study. The genotyping of control samples for the replication phase was done at the Laboratory of Neurogenetics of the National Institute on Aging, NIH (4594 [90%] of 5094) and at the core facility at the Institute of Child Health, UCL (500 [10%] of 5094). All control samples used in the replication phase were collected from the groups participating in the study (5094 samples passed quality control) and were of European ancestry from the following countries: France, Germany, Italy, the Netherlands, Spain, Sweden, the UK, and USA.

Investigators at every site obtained written informed consent from patients and control individuals. Every participating group provided consent for the use of these samples for the purposes of this study. Each study site obtained approval from a local ethics committee (UK ethics committee number 10/H0716/3) or institutional research ethics board.

Procedures

For every sample, 2 μg of DNA extracted from either blood or the brain at each collaborative site was collected (whole genome amplified DNA samples were excluded). Samples were securely stored at –20°C. Every sample was first screened for integrity and purity by means of gel electrophoresis on 1% agarose gel and concentrations were analysed by spectrophotometric (Nanodrop) or fluorometric (Qubit) quantification. The same procedure was implemented at NIH, UCL, and TTUHSC.

Samples from patients and control individuals included in the discovery phase were genotyped using Illumina human 370K, 550K, and 660K Quad Beadchips and Omni Express chips (Illumina Inc, CA, USA). We used Illumina NeuroX custom chips for all samples included in replication phase genotyping. The NeuroX chip is a partially custom-designed chip that specifically targets the main loci associated with several different neurological disorders obtained from GWAS or whole-exome sequencing data. The NeuroX chip holds about 267K SNPs, of which 3759 were FTD-specific, being selected from SNPs that had p values of less than 1×10^{-4} during the discovery phase of the study. These SNPs were tag SNPs based on European ancestry linkage-disequilibrium patterns from the most up-to-date data for samples of European ancestry from the 1000 Genomes project.[20] For all GWAS significant hits and candidate SNPs, five linkage-disequilibrium-based proxies or technical replicates were included on the array per locus, tagging associations within +/–250 kb and r^2 >0·5 from the most strongly associated proximal SNP. To replicate each locus, we picked the tag SNP most significant in the discovery phase; if no linkage-disequilibrium-based proxies were available, technical replicates were included. All genotyping arrays (discovery phase and replication phase) were assayed on the Illumina Infinium platform (Illumina, San Diego, CA, USA) at the Laboratory of Neurogenetics of the National Institute on Aging, NIH and at the core facility at the Institute of Child Health, UCL. All genotypes for this project were called centrally using Illumina Genome Studio and all 3759 SNPs of interest for FTD were manually examined to ensure high-quality genotype clusters before data export.

For the purpose of assessing possible biological relevance for any associated SNPs we used quantitative trait loci (QTL) data generated by the UK Brain Expression Consortium (UKBEC) and the North American Brain Expression Consortium (NABEC) for brain tissues assayed for genome-wide expression and methylation. Details about sample collection, RNA and DNA extraction, and genotyping are provided in the appendix.

Statistical analysis

We did standard quality control for GWAS data before association analyses. Briefly, for the discovery phase, we extracted overlapping SNPs across all Illumina arrays used. This was done as a means of dealing with the low numbers of matched cases and controls per study site or chip type to facilitate the FTD subtype analyses. We maximised sample size for the subtype analyses by pooling as many possible samples while sacrificing some array content, leaving 228 189 autosomal SNPs as a basis for imputation after the quality control was completed. We excluded samples possibly mismatched for sex by assessing X chromosome heterozygosity. Samples with a call rate of greater than 95% and SNPs with a minor allele frequency greater than 1% were filtered and included in the analyses. We calculated Hardy-Weinberg equilibrium p values (exclusion at p values $<1\times10^{-5}$). We assessed non-random missingness per SNP by case-control status with exclusion at p values of less than 1×10^{-5} and non-random missingness per SNP by haplotype at p values for exclusion $<1\times10^{-5}$. We assessed the presence of relatedness by identifying and excluding first-degree relatives (through identity by descent for any pairwise with an estimate of less than 0·125) and verified European ancestry by principal components analysis compared with HapMap3 populations, with European ancestry ascertained at values for the first two eigenvectors less than six SDs from the population mean for the combined Europeans from Utah (CEU) and Tuscans from Italy (TSI) reference samples.[21] After preliminary quality assessment, principal components analysis as implemented in EIGENSTRAT[22] was used to assess matching between cases and controls based on all available cases and controls. Custom coding in R (version 2.7) was used to match cases to controls. We treated each subtype (behavioural FTD, semantic dementia, progressive nonfluent aphasia, and FTD-MND) as a separate group in which the two most genetically similar unique controls per case were selected based on eigenvectors 1 and 2 to compensate for a lack of precisely matched controls at recruitment. In this respect, matched controls were unique per case and non-redundant across subtype datasets. Thus, cases and controls were matched for each subtype (behavioural FTD, semantic dementia, progressive nonfluent aphasia, and FTD-MND) based on similarity of the first two eigenvectors from principal components analysis and did not overlap across subtypes. We used logistic regression based on imputed dosages to assess the association between each SNP and any of the FTD subtypes, adjusting for eigenvectors 1 and 2 from principal components analysis as covariates. Eigenvectors were generated separately for each subtype and, as in the overall sample pool, parameter estimates for the first two were associated with case status at p values of less than 0·05. We did fixed-effects meta-analyses to combine results across subtypes and quantify heterogeneity across subtypes. Genomic inflation was minimal across subtypes and in the meta analysis across subtypes ($\lambda<1\cdot05$), therefore we did not use genomic control (see appendix for quantile-quantile plots and λ values per discovery phase analysis). SNPs were imputed to August, 2010 release of the 1000 Genomes haplotypes using default settings of minimac and were excluded if their minor allele frequency was less than 0·01 or imputation quality (Rsq) was less than 0·30 across all samples, leaving 6 026 385 SNPs for analyses. We used PLINK (version 1.07) for statistical analyses.

Weinberg College of Arts and Sciences, Northwestern University, IL, USA (Prof J Grafman); **Newcastle Brain Tissue Resource, Institute for Ageing and Health, Newcastle University, Newcastle upon Tyne, UK** (C M Morris, Prof J Attems MD, Prof T D Griffiths FMedSci); **Newcastle University, Institute for Ageing and Health, Campus for Ageing and Vitality, Newcastle upon Tyne, UK** (C M Morris, Prof J Attems MD, Prof A J Thomas PhD, E Jaros PhD); **Institute of Neuroscience, Newcastle University Medical School, Newcastle upon Tyne, UK** (C M Morris, Prof T D Griffiths); **Biomedical Research Building, Campus for Ageing and Vitality, Newcastle University, Newcastle upon Tyne, UK** (Prof I G McKeith MD); **Clinical Psychology Branch, Pisa University Hospital, Pisa, Italy, Laboratory of Clinical Biochemistry and Molecular Biology, University of Pisa, Pisa, Italy** (Prof P Pietrini MD); **Taub Institute, Departments of Psychiatry and Neurology, Columbia University, New York, NY, USA 10032** (E D Huey MD); **Behavioral Neurology Unit, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD, USA** (E M Wassermann MD, M C Tierney MSc);

	Behavioural variant frontotemporal dementia			Semantic dementia			Progressive nonfluent aphasia			Frontotemporal dementia with motor neuron disease			Frontotemporal lobar degeneration (unspecified)		
	Discovery phase	Replication phase	Total	Discovery phase	Replication phase	Total	Discovery phase	Replication phase	Total	Discovery phase	Replication phase	Total	Discovery phase	Replication phase	Total
Australia*	NA	56	56	NA	26	26	NA	19	19	NA	20	20	NA	0	0
Belgium	135	27	162	13	1	14	22	2	24	21	2	23	0	10	10
Canada	22	5	27	1	1	2	0	5	5	1	7	8	0	11	11
Denmark	2	NA	2	0	NA	0	1	NA	1	4	NA	4	0	NA	0
France	135	30	165	3	0	3	8	3	11	59	8	67	0	1	1
Germany	209	18	227	45	8	53	55	6	61	11	1	12	0	0	0
Italy	443	186	629	28	22	50	69	86	155	24	16	40	0	61	61
Netherlands	159	37	196	47	31	78	24	6	30	20	3	23	0	0	0
Spain	NA	194	194	NA	41	41	NA	51	51	NA	13	13	NA	10	10
Sweden	7	53	60	2	20	22	6	10	16	3	8	11	0	7	7
UK*	207	152	359	75	53	128	69	44	113	50	16	66	0	19	19
USA	315	25	340	147	12	159	81	15	96	36	21	57	0	102	102
Total	1634 (1377†)	783 (690†)	2417 (2061†)	361 (308†)	215 (190†)	576 (495†)	335 (269†)	247 (221†)	582 (486†)	229 (200†)	115 (94†)	344 (294†)	0	221 (177†)	221 (177†)

NA=not applicable. *Used the same control samples. †The number of the samples that passed genotyping data quality control and were used for association analyses.

Table 2: **Sample characteristics, by subtype**

Neuropathology Department, Walton Centre FT, Liverpool, UK (A Baborie MD); **Neuropathology/Cellular Pathology, Royal Victoria Infirmary, Newcastle upon Tyne, UK** (E Jaros); **Neurogenetics Laboratory, Division of Neurosciences, Center for Applied Medical Research, Universidad de Navarra, Pamplona, Spain** (P Pastor, C Razquin PhD, S Ortega-Cubero, E Alonso BSc); **Department of Neurology, Clínica Universidad de Navarra, University of Navarra School of Medicine, Pamplona, Spain** (P Pastor); **Neuroepidemiology and Ageing Research Unit, School of Public Health, Faculty of Medicine, The Imperial College of Science, Technology and Medicine, London, UK** (R Perneczky MD); **West London Cognitive Disorders Treatment and Research Unit, West London Mental Health Trust, London TW8 8 DS, UK** (R Perneczky); **Department of Psychiatry and Psychotherapy, Technische Universität München, Munich, Germany** (R Perneczky, J Diehl-Schmid MD, P Alexopoulos MD, A Kurz MD); **Neurology I, Department of Neuroscience, University of Torino, Italy, AO Città della Salute e della Scienza di Torino, Italy** (I Rainero MD, E Rubino MD, Prof L Pinessi MD); **Tanz Centre for Research in Neurodegenerative Diseases and Department of Medicine, University of Toronto, Toronto, Ontario, Canada** (E Rogaeva PhD, P St George-Hyslop MD); **Cambridge Institute for Medical Research and the Department of Clinical Neurosciences, University of Cambridge, Cambridge, UK** (P St George-Hyslop); **Division of Neurology V and Neuropathology, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano, Italy** (G Rossi PhD, F Tagliavini MD, G Giaccone MD); **Cambridge University Department of Clinical Neurosciences, Cambridge CB2 0SZ, UK** (J B Rowe PhD); **MRC Cognition and Brain Sciences Unit, Cambridge, UK** (J B Rowe); **Behavioural and Clinical Neuroscience Institute, Cambridge, UK** (J B Rowe); **Department of Psychiatry and Psychotherapy, University of Freiburg Medical School, Germany**

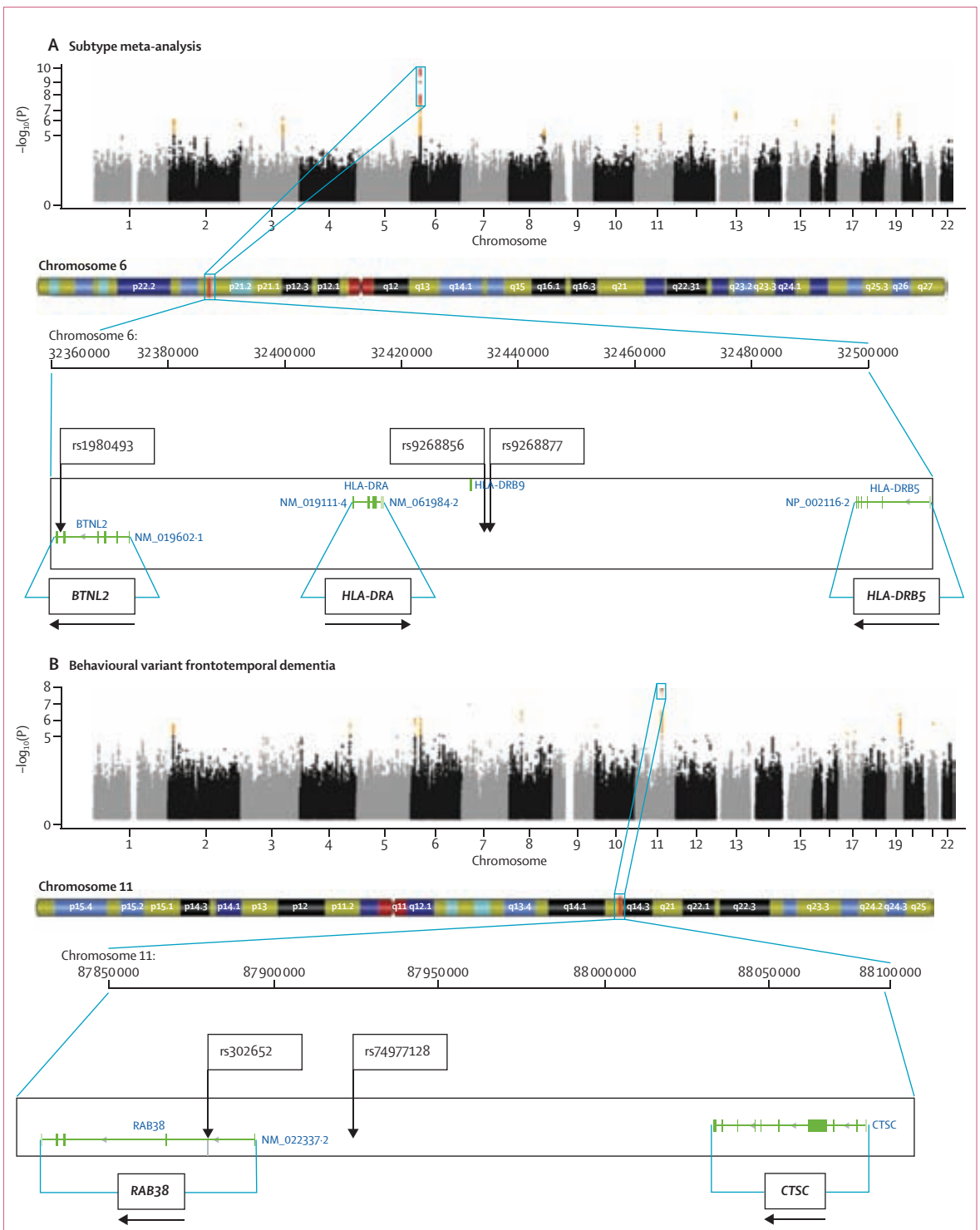


Figure 1: **Manhattan plots identifying regions with genome-wide significant associations**
(A) Single–nucleotide polymorphisms (SNPs) with genome-wide significant p values ($p<5\times10^{-8}$) are depicted as red dots and locate to 6p21.3. The associated SNPs map to intron 5 of *BTNL2* and to the intergenic region between *HLA-DRA* and *HLA-DRB*. (B) Manhattan plot for the behavioural variant frontotemporal dementia subtype in the discovery phase. SNPs with genome-wide significant p values ($p<5\times10^{-8}$) are depicted as red dots and locate to 11q14. The associated SNPs map to intron 1 of *RAB38* and to the intergenic region between *RAB38* and *CTSC*.

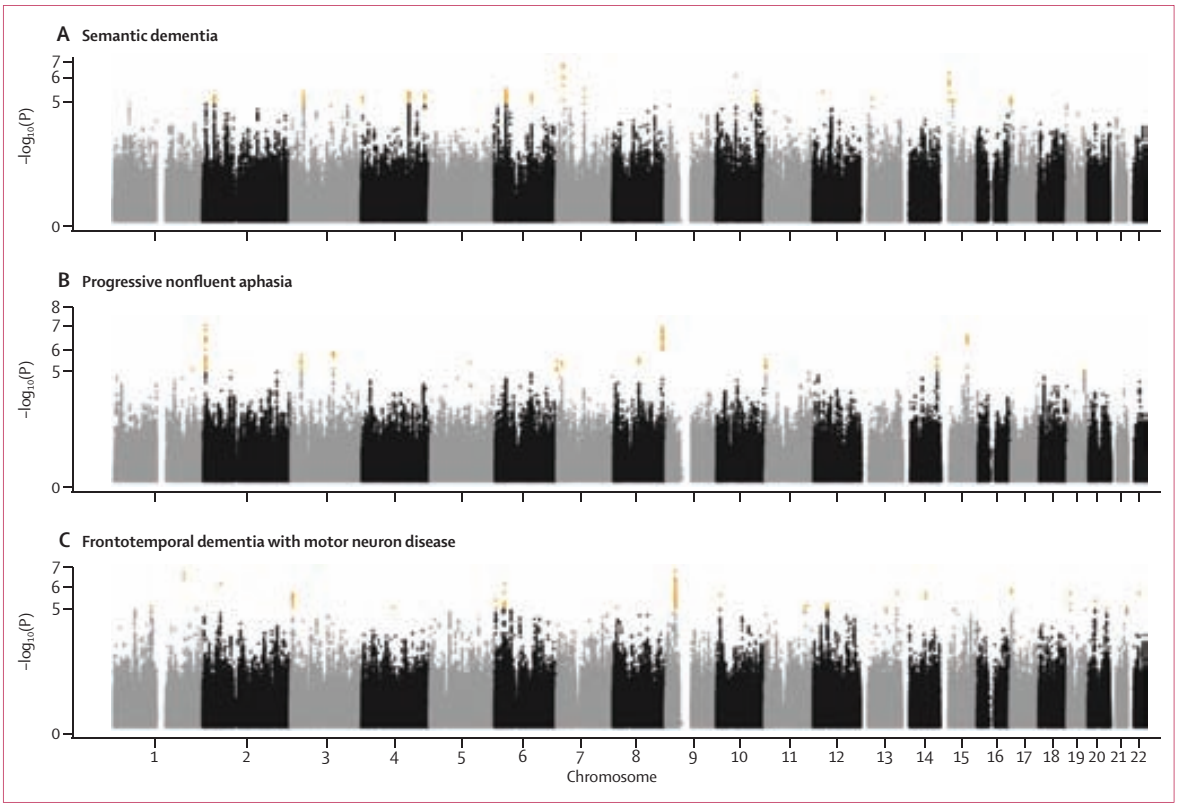


Figure 2: **Manhattan plots identifying regions with suggestive associations**
Manhattan plots for semantic dementia (A), progressive nonfluent aphasia (B), and frontotemporal dementia with motor neuron disease (C).

For the replication phase, we did standard quality control as for the discovery phase with slight adjustments to account for the bias in NeuroX array content (candidate neurological or neurodegenerative disease SNPs and exonic content). Standard content variants included on the NeuroX array that were used for sample quality control were called using a publicly available cluster file based on more than 60 000 samples.[23] See the appendix for details about QTL statistical analysis.[24–37] For quality control, variants with GenTrain scores greater than 0·70 (indicative of high-quality genotype clusters) were extracted first to calculate call rates. Samples with call rates greater than 95% were excluded, as were samples whose genetically determined sex conflicted with that from the clinical data and samples exhibiting excess heterozygosity. Next, SNPs overlapping with HapMap phase 3 samples were extracted from the previous subset and pruned for linkage disequilibrium (SNPs excluded if r^2 >0·50 within a 50 SNP sliding window), and SNPs with minor allele frequency less than 5%, Hardy-Weinberg equilibrium p values less than 1×10^{-5}, and per SNP missingness rates greater than 5%. At this stage, we used pairwise identity-by-descent filtering to remove samples that were cryptically related and principal components analysis to identify samples to be excluded when genetic ancestry was not consistent with European descent based on comparisons with HapMap phase 3 reference populations. For replication analyses and due to an effort to maximise the restricted power of this phase compared to the discovery phase, analyses of each subtype included all control samples available, adjusting for the first five eigenvectors only from principal components analysis as covariates in the logistic regression model. No other adjustments were implemented. Additionally, we pooled the individual genotypes from different subsets in the replication phase to help increase statistical power. For details about QTL statistical analysis, see appendix. This study used the high-performance computational capabilities of the Biowulf Linux cluster at the NIH.

Role of the funding source

The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. No pharmaceutical company or other agency paid to write this Article. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

In the discovery phase, we analysed samples from 2154 patients (table 1) and 4308 controls. We first did

(J C M Schlachetzki MD); **Department of Molecular Neurology, University Hospital Erlangen, Erlangen, Germany** (J C M Schlachetzki); **MRC Prion Unit, Department of Neurodegenerative Disease, UCL Institute of Neurology, London, UK** (J Uphill BSc, Prof J Collinge MD, Prof S Mead PhD); **Neurologische Klinik und Poliklinik, Ludwig-Maximilians-Universität, Munich, Germany** (A Danek MD); **German Center for Neurodegenerative Diseases (DZNE), Munich, Germany** (A Danek); **University of Pennsylvania Perelman School of Medicine, Department of Neurology and Penn Frontotemporal Degeneration Center, Philadelphia, PA, USA** (V M Van Deerlin PhD, Prof M Grossman MD, Prof J Q Trojanowski PhD); **Neurodegenerative Brain Diseases group, Department of Molecular Genetics, VIB, Antwerp, Belgium** (J van der Zee PhD, W Deschamps MSc, T Van Langenhove MD, M Cruts PhD, Prof C Van Broeckhoven PhD); **Laboratory of Neurogenetics, Institute Born-Bunge, University of Antwerp, Antwerp, Belgium** (J van der Zee, W Deschamps, T Van Langenhove, M Cruts, Prof C Van Broeckhoven); **Neurorehabilitation Unit, Deptartment Of Clinical Neuroscience, Vita-Salute University and San Raffaele Scientific Institute, Milan, Italy** (Prof S F Cappa MD); **Inserm, UMR_S975, CRICM, F-75013; UPMC Univ Paris 06, UMR_S975, F-75013; and CNRS UMR 7225, F-75013, Paris, France** (I Le Ber MD, A Brice MD); **AP-HP, Hôpital de la Salpêtrière, Département de Neurologie-Centre de Références des Démences Rares, F-75013, Paris, France** (I Le Ber, A Brice); **Service de Neurologie, Inserm U1079, CNR-MAJ, Rouen University Hospital, France** (Prof D Hannequin MD); **Service de neurologie, CH Saint Brieuc, France** (V Golfier MD); **Service de Neurologie, CHU Nantes, France** (M Vercelletto MD); **Department of Neurosciences, Psychology, Drug Research and Child Health (NEUROFARBA) University of Florence, Florence, Italy** (B Nacmias PhD, Prof S Sorbi PhD, S Bagnoli PhD, I Piaceri PhD);

Danish Dementia Research Centre, Neurogenetics Clinic, Department of Neurology, Rigshospitalet, Copenhagen University Hospital, Denmark (J E Nielsen MD, L E Hjermind MD); **Department of Cellular and Molecular Medicine, Section of Neurogenetics, The Panum Institute, University of Copenhagen, Denmark** (J E Nielsen, L E Hjermind); **Saarland University Hospital, Department for Psychiatry and Psychotherapy, Homburg/Saar, Germany** (Prof M Riemenschneider MD); **Saarland University, Laboratory for Neurogenetics, Kirrberger, Homburg/Saar, Germany** (Prof M Riemenschneider, M Mayhaus PhD, G Gasparoni PhD, S Pichler MSc, W Gu PhD); **University Regensburg, Department of Psychiatry, Psychotherapy and**

separate association analyses for each subtype (behavioural FTD, semantic dementia, progressive nonfluent aphasia, and FTD-MND; table 2) and then undertook a meta-analysis of the entire dataset. Findings from the meta-analysis showed 29 SNPs (appendix) exceeding genome-wide significance (p value $<5\times10^{-8}$) at the *HLA* locus (6p21.3), encompassing the butyrophilin-like 2 (MHC class II associated) gene (*BTNL2*) and the major histocompatibility complex, class II, DR alpha (*HLA-DRA*), and DR beta 5 (*HLA-DRB5*; figure 1, table 3). To identify susceptibility loci for the behavioural FTD subtype we analysed 1377 patient samples (table 2) and 2754 control samples. Two non-coding SNPs at 11q14, locating to intron 1 of the gene *RAB38*, member RAS oncogene family (*RAB38*; rs302652) and encompassing *RAB38* and cathepsin C (*CTSC*; rs74977128), passed the genome-wide significance threshold (figure 1, table 3). Similarly, we did analyses on the other subtypes (table 1): 308 semantic dementia versus 616 controls, 269 progressive nonfluent aphasia versus 538 controls, and 200 FTD-MND versus 400 controls. No SNP reached genome-wide significance in either subtype, probably due to the small sample size. However, several SNPs (appendix) showed suggestive associations (p values between 1×10^{-6} and 1×10^{-7}; figure 2) and warrant further investigation in future screenings.

In the replication phase, we analysed samples from 1372 patients (table 1) and 5094 controls. We assessed the associated SNPs at 6p21.3 (rs9268877, rs9268856, and rs1980493) in the whole replication cohort (table 3). Table 3 shows findings from the surrogate or proxy SNPs assessed for replication at 11q14 in 690 behavioural FTD cases: rs302668 and rs16913634. Combined analyses of discovery and replication phases showed genome-wide significant association at 6p21.3 for all SNPs (table 3). Joint p values of the SNPs at 11q14 only revealed suggestive association for rs302668 (table 3) possibly because of decreased power due to proxy-based replication (r^2 of rs302652 to rs302668=0·65).

We then assessed biological relevance for the novel potential loci in human brain cortex tissues assayed for genome-wide expression and methylation. There was no eQTL in our dataset, but assessment of Zeller and colleagues' dataset[38] showed a *cis*-eQTL ($p=5\cdot05\times10^{-32}$;

	Chromosome	Base pair	Candidate gene	Minor allele	Major allele	Frequency of minor allele (r^2 when applicable)	Imputation quality	Odds ratio (95% CI)	Standard error	p value
Discovery phase										
Behavioural variant frontotemporal dementia										
rs302652	11	87894831	*RAB38*	A	T	0·259	0·9296	0·730 (0·65–0·82)	0·057	$2\cdot02\times10^{-8}$
rs74977128	11	87936874	*RAB38/CTSC*	C	T	0·118	0·4182	1·815 (1·48–2·24)	0·107	$3\cdot06\times10^{-8}$
All frontotemporal dementia*										
rs9268877	6	32431147	*HLA-DRA/HLA-DRB5*	A	G	0·440	0·7783	1·331 (1·22–1·45)	0·045	$1\cdot65\times10^{-10}$
rs9268856	6	32429719	*HLA-DRA/HLA-DRB5*	A	C	0·251	0·8563	0·752 (0·68–0·83)	0·050	$1\cdot30\times10^{-8}$
rs1980493	6	32363215	*BTNL2*	C	T	0·147	0·9642	0·720 (0·69–0·81)	0·060	$4\cdot94\times10^{-8}$
Replication phase										
Behavioural variant frontotemporal dementia										
rs302668 (proxy)	11	87876911	*RAB38*	C	T	0·325 (0·65)	NA	0·877 (0·77–0·99)	0·064	0·041
rs16913634 (proxy)	11	87934068	*RAB38/CTSC*	A	G	0·104 (0·54)	NA	0·964 (0·79–1·17)	0·098	0·710
All frontotemporal dementia*										
rs9268877	6	32431147	*HLA-DRA/HLA-DRB5*	A	G	0·449	NA	1·080 (0·98–1·18)	0·047	0·104
rs9268856	6	32429719	*HLA-DRA/HLA-DRB5*	A	C	0·253	NA	0·878 (0·79–0·97)	0·053	0·014
rs1980493	6	32363215	*BTNL2*	C	T	0·145	NA	0·85 (0·75–0·97)	0·068	0·020
Discovery and replication combined										
Behavioural variant frontotemporal dementia										
rs302668 (proxy)	11	87876911	*RAB38*	C	T	0·292 (0·65)	NA	0·814 (0·71–0·92)	0·064	$2\cdot44\times10^{-7}$
rs16913634 (proxy)†	11	87934068	*RAB38/CTSC*	A	G	0·111 (0·54)	NA	1·248 (1·14–1·37)	0·049	$8\cdot15\times10^{-4}$
All frontotemporal dementia*										
rs9268877†	6	32431147	*HLA-DRA/HLA-DRB5*	A	G	0·4445	NA	1·204 (1·11–1·30)	0·039	$1\cdot05\times10^{-8}$
rs9268856	6	32429719	*HLA-DRA/HLA-DRB5*	A	C	0·252	NA	0·809 (0·76–0·86)	0·029	$5\cdot51\times10^{-9}$
rs1980493	6	32363215	*BTNL2*	C	T	0·146	NA	0·775 (0·69–0·86)	0·058	$1\cdot57\times10^{-8}$

Replication and joint analyses were assessed for the same single-nucleotide polymorphisms (SNPs) at 6p21.3, whereas proxy SNPs were used to assess the association at 11q14 (for which r^2 values are included). The odds ratio is shown for the minor allele. NA=not applicable. *Denotes only minimal cross-subtype heterogeneity, with heterogeneity p values ranging from 0·793 to 0·944 based on Cochran's Q test. †Heterogeneity p value <0·01 in the meta-analyses of the discovery and replication phases combined.

Table 3: **Characteristics of single-nucleotide polymorphisms exceeding genome-wide significance in the discovery phase**

	CpG probe	Single-nucleotide polymorphism	Chromosome	Position (base pair)	Reference allele	Alternate allele	Frequency of reference allele	Imputation quality	Effect estimate of alternate allele (in Z units)	Standard error	p value	FDR adjsuted p value	Probe start (base pair)	Probe end (base pair)	Symbol
Frontal cortex (CpG methylation)	cg21415604	rs1980493	6	32363215	T	C	0·8361	0·9888	−0·463	0·116	0·0000701	0·00834666	31948433	31948483	*C4B*
Frontal cortex (CpG methylation)	cg25764570	rs1980493	6	32363215	T	C	0·8361	0·9888	−0·652	0·116	$2·17\times10^{-8}$	0·00000773	32407239	32407289	*HLA-DRA*
Frontal cortex (CpG methylation)	cg25764570	rs9268856	6	32429719	C	A	0·748	0·9687	−0·484	0·1	$1·16\times10^{-6}$	0·000207417	32407239	32407289	*HLA-DRA*

Association is shown for rs1980493 and rs9268877, suggestive of their implication in methylation processes and patterns in relation to *HLA-DRA*.

Table 4: **Summary of association of top hits with *cis*-methylation levels at 6p21.3**

	Reference allele	Alternate allele	Previous studies			This study (discovery phase)										Frontotemporal dementia with motor neuron disease	
			Frequency of reference allele	Reported association		Frequency of reference allele	Imputation quality (RSQ)	Meta-analysis (all frontotemporal dementia)		Behavioural variant frontotemporal dementia		Semantic dementia		Progressive nonfluent aphasia			
				p value (joint)	Odds ratio (95% CI)			p value	Odds ratio (95% CI)	p value	Odds ratio (95% CI)	p value	Odds ratio (95% CI)	p value	Odds ratio (95% CI)	p value	Odds ratio (95% CI)
Chromosome 17																	
Progressive supranuclear palsy or corticobasal degeneration[40]																	
MAPT																	
rs8070723 (44081064)	A	G	0·950 (stage 1); 0·940 (stage 2)	$1·50\times10^{-116}$	5·46 (4·72–6·31)	0·765	0·8400	$2·80\times10^{-4}$	1·201 (1·09–1·32)	$3·14\times10^{-3}$	1·201 (1·06–1·36)	$4·34\times10^{-1}$	1·103 (0·86–1·41)	$8·72\times10^{-3}$	1·471 (1·10–1·97)	$5·82\times10^{-1}$	1·091 (0·80–1·49)
rs242557 (44019712)	G	A	0·470 (stage 1); 0·500 (stage 2)	$4·20\times10^{-70}$	0·51 (0·47–0·55)	0·634	0·5246	$4·82\times10^{-3}$	0·853 (1·05–1·31)	$1·27\times10^{-2}$	0·841 (0·73–0·96)	$3·20\times10^{-1}$	0·867 (0·65–1·15)	$2·02\times10^{-1}$	0·815 (0·59–1·11)	$8·23\times10^{-1}$	0·961 (0·68–1·36)
Chromosome 19																	
Alzheimer's disease[41]																	
TOMM40/APOE																	
rs2075650 (45395619)	G	A	0·150	$1·04\times10^{-295}$	2·53 (2·41–2·66)	0·141	0·9978	$8·81\times10^{-7}$	1·304 (0·69–0·85)	$1·37\times10^{-6}$	1·383 (0·63–0·82)	$3·64\times10^{-2}$	1·326 (0·58–0·98)	$8·69\times10^{-1}$	0·976 (0·76–1·38)	$2·06\times10^{-1}$	1·252 (0·56–1·13)

(Table 5 continues on next page)

Psychosomatics, Universitätsstr 84, Regensburg, Germany (B Ibach PhD); **Luxembourg Centre For Systems Biomedicine (LCSB), University of Luxembourg, Luxembourg** (W Gu); **Dementia Research Centre, Department of Neurodegenerative Disease, UCL Institute of Neurology, Queen Square, London, UK** (J D Rohrer, Prof M N Rossor MD, Prof N C Fox MD, J D Warren PhD); **University of Cambridge, Department of Clinical Neurosciences, John Van Geest Brain Repair Centre, Cambridge, UK** (Prof M G Spillantini PhD); **MRC Centre for Neuropsychiatric Genetics and Genomics, Cardiff University, School of Medicine, Cardiff, UK** (Prof H R Morris PhD); **German Center of Neurodegenerative Diseases-Tübingen, Tübingen, Germany** (P Rizzu PhD, Prof P Heutink PhD); **Salford Royal Foundation Trust, Faculty of Medical and Human Sciences, University of Manchester, UK** (A Richardson MB); **Institute of Brain, Behaviour and Mental Health, The University of Manchester, Withington, Manchester, UK** (A Gerhard MD); **Regional Neurogenetic Centre, ASPCZ, Lamezia Terme, Italy** (A C Bruni MD, R Maletta MD, F Frangipane MD, C Cupidi MD, L Bernardi PhD, M Anfossi PhD, M Gallo PhD, M Elena Conidi PhD, N Smirne BSc); **Department of Neuroscience** (Prof R Rademakers PhD, M Baker BSc, Prof D W Dickson MD), **Department of Neurology** (Prof N R Graff-Radford MD), **Mayo Clinic Jacksonville, Jacksonville, FL, USA; Department of Neurology** (Prof R C Petersen MD, Prof D Knopman MD, Prof K A Josephs MD, Prof B F Boeve MD), **Department of Pathology** (Prof J E Parisi MD), **Mayo Clinic Rochester, Rochester, MN, USA; Department of Neurology** (W W Seeley MD, Prof B L Miller MD, A M Karydas BA, Prof H Rosen MD), **University of California, San Francisco, CA, USA; Department of Neurology, Erasmus Medical Centre, Rotterdam, The Netherlands** (Prof J C van Swieten MD, E G P Dopper MD, H Seelaar PhD); **Department of Medical Genetics, VU University Medical**

	Reference allele	Alternate allele	Previous studies			This study (discovery phase)										
			Frequency of reference allele	Reported association		Frequency of reference allele	Imputation quality (RSQ)	Meta-analysis (all frontotemporal dementia)		Behavioural variant frontotemporal dementia		Semantic dementia		Progressive nonfluent aphasia		Frontotemporal dementia / motor neuron disease	
				p value (joint)	Odds ratio (95% CI)			p value	Odds ratio (95% CI)	p value	Odds ratio (95% CI)	p value	Odds ratio (95% CI)	p value	Odds ratio (95% CI)	p value	Odds ratio (95% CI)
(Continued from previous page)																	
Chromosome 9																	
Amyotrophic lateral sclerosis[39]																	
C9orf72/MOB3B																	
rs3849942 (27543281)	A	G	0·260	$1·01\times10^{-8}$	1·23 (NA)	0·253	0·9996	$4·38\times10^{-4}$	1·166 (1·07–1·27)	$7·38\times10^{-3}$	1·155 (0·78–0·96)	$9·89\times10^{-1}$	1·010 (0·80–1·25)	$9·03\times10^{-1}$	0·990 (0·79–1·31)	$2·12\times10^{-6}$	1·957 (0·39–0·68)
Chromosome 7																	
FTLD-TDP[17]																	
TMEM106B																	
rs1990622 (12283787)	A	G	0·679	$1·08\times10^{-11}$	1·64 (1·41–1·89)	0·600	0·9588	$7·88\times10^{-2}$	1·080 (0·99–1·16)	$5·85\times10^{-3}$	1·144 (1·04–1·26)	$8·36\times10^{-1}$	0·978 (0·80–1·20)	$8·98\times10^{-1}$	0·985 (0·79–1·23)	$3·11\times10^{-1}$	0·876 (0·68–1·13)
rs6966915 (12265988)	C	T	0·679	$1·63\times10^{-11}$	1·64 (1·41–1·89)	0·596	0·9675	$1·21\times10^{-1}$	1·070 (0·87–1·02)	$5·74\times10^{-3}$	1·144 (1·04–1·26)	$5·27\times10^{-1}$	0·936 (0·76–1·15)	$7·26\times10^{-1}$	0·961 (0·77–1·20)	$3·62\times10^{-1}$	0·888 (0·69–1·14)
rs1020004 (12255778)	T	C	0·767	$5·00\times10^{-11}$	1·66 (1·43–1·96	0·693	0·9538	$4·59\times10^{-1}$	1·030 (0·95–1·12)	$5·71\times10^{-2}$	1·104 (1·00–1·22)	$8·53\times10^{-1}$	0·980 (0·79–1·21)	$5·00\times10^{-1}$	0·921 (0·72–1·17)	$1·20\times10^{-1}$	0·805 (0·61–1·06)
Chromosome 11																	
Multiple sclerosis[44,45]																	
RAB38																	
rs1386330 (87819427)	C	T	0·130	$2·00\times10^{-6}$	NA	0·141	0·9694	$3·35\times10^{-1}$	1·050 (0·85–1·06)	$6·09\times10^{-1}$	1·040 (0·84–1·10)	$7·60\times10^{-1}$	1·040 (0·72–1·27)	$6·97\times10^{-1}$	1·060 (0·68–1·29)	$3·00\times10^{-1}$	0·829 (0·58–1·18)
Chromosome 6																	
Multiple sclerosis[43-45]																	
HLA-DRA																	
rs3135388 (32413051)	A	G	0·230	$8·94\times10^{-81}$	1·99 (1·84–2·15)	0·131	0·9734	$4·80\times10^{-2}$	1·122 (1·00–1·26)	$2·10\times10^{-1}$	1·095 (0·79–1·05)	$1·25\times10^{-1}$	1·254 (0·60–1·06)	$5·02\times10^{-1}$	1·120 (0·64–1·24)	$6·10\times10^{-1}$	1·105 (0·61–1·33)
rs3129871 (32406342)	A	C	0·504	$5·70\times10^{-15}$	1·72 (1·59–1·86)	0·337	0·9379	$3·43\times10^{-1}$	0·961 (0·88–1·04)	$3·15\times10^{-1}$	0·949 (0·95–1·16)	$4·94\times10^{-1}$	1·078 (0·75–1·15)	$8·24\times10^{-1}$	0·974 (0·81–1·30)	$2·72\times10^{-1}$	0·859 (0·89–1·53)
Parkinson's disease[46]																	
HLA-DRA																	
rs3129882 (32409530)	G	A	0·450	$1·90\times10^{-10}$	1·26 (1·17–1·35)	0·456	0·9992	$3·36\times10^{-2}$	1·086 (0·85–0·99)	$3·27\times10^{-2}$	1·106 (0·82–0·99)	$7·52\times10^{-1}$	1·033 (0·79–1·18)	$5·74\times10^{-1}$	1·065 (0·75–1·17)	$7·07\times10^{-1}$	1·049 (0·74–1·22)

NA=not applicable. FLTD-TDP=frontotemporal lobar degeneration with TDP43-positive inclusions. RSQ=r^2 imputation quality coefficient.

Table 5: **Bidirectional analysis of single-nucleotide polymorphisms and loci associated with other neurodegenerative disorders and our study population**

appendix) at 11q14 for rs302652 (chr11:87894881, risk allele T) causing a decreased expression of *RAB38* (Illumina ILMN_2134974 located on chr11:87846656-87846705) in monocytes. These data suggest a role in transcriptional processes *in cis* for this SNP. Furthermore, we identified significant *cis*-mQTL at 6p21.3 after multiple test correction for rs1980493 (risk allele T) that associated with changes in the methylation levels related to *HLA-DRA* in the frontal cortex (table 4).

To assess potential genetic overlap between FTD and closely related forms of neurodegenerative diseases we selected relevant SNPs for candidate loci and analysed them in our dataset. This analysis included published association studies for amyotrophic lateral sclerosis,[39] progressive supranuclear palsy and corticobasal degeneration,[40] Alzheimer's disease,[41] and FTLD-TDP.[17] We also assessed whether the two loci identified through this study had also been reported previously in other studies of neurological disorders.

For the *C9orf72* locus (for amyotrophic lateral sclerosis), the SNP rs3849942 achieved a p value of $2{\cdot}12\times10^{-6}$ and an OR of 1·957 in the FTD-MND subtype consistent with our post-hoc analyses (about 23% of expansion carriers in this subtype; table 5, appendix). Association was modest in behavioural FTD ($p=7{\cdot}38\times10^{-3}$; OR=1·155) as well as in the entire discovery cohort ($p=4{\cdot}38\times10^{-4}$; OR=1·166), while there was no evidence for association in the semantic dementia or progressive nonfluent aphasia subtypes (table 5). These results confirm that the *C9orf72* locus associates mainly with FTD-MND and to a lesser extent with behavioural FTD (appendix).

For the *MAPT* locus (PSP/CBD), the SNPs rs242557 and rs8070723[40] reached modest p values between 10^{-3} and 10^{-4} only in the entire cohort and in the behavioural FTD and progressive nonfluent aphasia subtypes (rs8070723 only; table 5). The effect was small in our study although in the same direction as in the GWAS for progressive supranuclear palsy (5·46[40] *vs* about 1·2–1·4 in our study; table 5). These results reflect the fact that we excluded all known chromosome 17 mutation carriers and that tau pathology was a less common feature within our study population.

For the *TOMM40*/*APOE* locus (Alzheimer's disease), the SNP rs2075650 reached a p value of $8{\cdot}81\times10^{-7}$ in the entire dataset and $1{\cdot}37\times10^{-6}$ in behavioural FTD, whereas the semantic dementia, progressive nonfluent aphasia, and FTD-MND subtype p values were in the range of 10^{-1} and 10^{-2} (table 5). Several Alzheimer's disease GWASs reported association with the minor allele of this SNP with ORs greater than 2·5,[41] but in our study the OR was about 1·3 (table 5). This suggestive association might reflect clinical overlap (about 15%) between patients with clinically diagnosed FTD and those with Alzheimer's disease.[42]

For the *TMEM106B* locus (FTLD-TDP), we assessed the three associated SNPs reported by Van Deerlin and colleagues (rs1990622; rs6966915; rs1020004).[17] All achieved modest p values in the entire dataset with lowest p values in the range of 10^{-2}–10^{-3} only in the behavioural FTD subtype (table 5). Van Deerlin and colleagues' study[17] was done on samples from patients with autopsy-confirmed FTLD-TDP, whereas our cohort is mainly clinically defined. Additionally, the previous study included many *GRN* mutation carriers, who frequently present with behavioural FTD;[17] in our study, *GRN* mutation carriers were excluded. Biochemical evidence has suggested that *TMEM106B* is directly related to *GRN* metabolism,[13] thus we regard our data as a limited replication of the original finding (ie, they do not substantiate earlier findings).

Finally, the *RAB38* locus previously showed suggestive association in multiple sclerosis,[43] whereas the *HLA* locus was reported to associate with multiple sclerosis,[44,45] Parkinson's disease,[19,46] and Alzheimer's disease.[47] None of the SNPs reported in these studies, and which were assessed in our dataset (table 5),[43–46] showed association with FTD, probably suggesting that different risk haplotype sub-structures at the same loci associate with distinctive phenotypes.

Discussion

We have identified two novel potential loci for FTD: 6p21.3, encompassing the *HLA* locus (immune system), and 11q14, encompassing *RAB38*/*CTSC* (transcripts of which are related to lysosomal biology). Our data suggest that these loci might affect expression and methylation in *cis* and indicate that immune system processes, and possibly lysosomal and autophagy pathways, are potentially involved in the pathogenesis of FTD.

FTD is characterised by a broad range of clinical manifestations, differential pathological signatures, and substantial genetic variability, which imply complex disease mechanisms.[15] In the search for novel disease risk loci associated with FTD we have done an extensive GWAS on a large cohort of mainly clinically diagnosed FTD samples from patients of European ancestry. Several limitations might apply to this study. In view of the phenotype heterogeneity of FTD, and considering that it is a rare neurodegenerative disorder,[2] testing the hypothesis "common variant, common disease" for diseases of this kind is challenging and clearly benefits from large sample sizes. Additionally, our findings might indicate association with specific loci without necessarily implying causality; low heritability due to common variability might also apply. However, the QQ plots and associated λ values (appendix) conformed to GWAS standards, lending support to our findings.

We included samples from more than 3500 patients and, thus, we know of no larger GWAS for FTD. We have identified two novel potential loci for FTD: 11q14, encompassing *RAB38*/*CTSC*, was suggestive for the behavioural FTD subtype, and 6p21.3, encompassing the *HLA* locus was statistically significant for the entire cohort.

Centre, Amsterdam, The Netherlands (Prof J C van Swieten); **Alzheimer Centre and Department of Neurology, VU University Medical Centre, Amsterdam, The Netherlands** (Y A L Pijnenburg MD, Prof P Scheltens MD); **Department of Basic Medical Sciences, Neurosciences and Sense Organs of the Aldo Moro, University of Bari, Italy** (G Logroscino MD, R Capozzo MD); **Department of Medical and Molecular Genetics, King's College London, Guy's Hospital, London, UK** (A Ramasamy); **Department of Molecular Cardiology, IRCCS Fondazione S Maugeri, Pavia, Italy** (V Novelli PhD); **Cardiovascular Research Unit, IRCCS Multimedica, Milan, Italy** (An A Puca MD); **Department of Medicine and Surgery, University of Salerno, Baronissi (SA), Italy** (A A Puca); **Neurology Department, IRCCS Multimedica, Milan, Italy** (M Franceschi MD); **Department of Clinical Medicine and Surgery, University of Naples Federico II, Naples, Italy** (Prof A Postiglione MD); **Geriatric Center Frullone-ASL Napoli 1 Centro, Naples, Italy** (G Milan MD, P Sorrentino MD); **UCL Genomics, Institute of Child Health (ICH), UCL, London, UK** (M Kristiansen PhD); **Karolinska Institutet, Department NVS, KI-Alzheimer Disease Research Center, Stockholm, Sweden** (H-H Chiang PhD, Prof C Graff MD); **Department of Geriatric Medicine, Genetics Unit, Karolinska Universtiy Hospital, Stockholm** (H-H Chiang, Prof C Graff); **Université Lille Nord de France, Lille, France** (Prof F Pasquier MD, A Rollin MD, V Deramecourt MD, F Lebert MD); **and Laboratory of Neurosciences, National Institute on Aging, National Institutes of Health, Bethesda, MD, USA** (D Kapogiannis MD)

Correspondence to:
Prof John Hardy, Reta Lila Weston Research Laboratories, Department of Molecular Neuroscience, University College London Institute of Neurology, London WC1N 3BG, UK
j.hardy@ucl.ac.uk

See **Online** for appendix

For more on **Biowulf** see http://biowulf.nih.gov

For more on the **expression of *RAB38*** see http://www.genecards.org/cgi-bin/carddisp.pl?gene=RAB38

Panel: Research in context

Systematic review

We searched PubMed for research and review articles on frontotemporal dementia using the following terms: "frontotemporal dementia AND genetics" and "frontotemporal dementia AND review".[1,4,5,8–17,54] We compared our results to several previously published genome-wide association studies. We identified only one directly relevant study that investigated a pathologically defined subtype of frontotemporal dementia (frontotemporal lobar degeneration with TDP43-positive inclusions; FTLD-TDP).[17] The other studies were of related diseases such as amyotrophic lateral sclerosis,[39] Alzheimer's disease,[41,47] progressive supranuclear palsy and corticobasal degeneration,[40] multiple sclerosis,[43–45] and Parkinson's disease.[19,46]

Interpretation

To the best of our knowledge, ours is the first genome-wide association study in samples from patients with clinical frontotemporal dementia. In view of the complexity and heterogeneity of the disease, mutations in only three main genes—*MAPT*, *GRN*, and *C9orf72*—have been associated with frontotemporal dementia, and these explain only a small proportion of cases. Most importantly, little is known about the mechanisms involved in the development of this disorder. Our findings suggest that common variability in loci that point to immune processes and possibly to lysosomal biology and autophagy are involved in the pathobiology of the disease. These findings provide a basis for future replication and functional studies.

RAB38[48] encodes the transmembrane protein RAB38, which is expressed in the thyroid, in elements of the immune system, and in the brain. From a functional perspective, RAB38 has been shown to mediate protein trafficking to lysosomal-related organelles and maturation of phagosomes.[49,50] CTSC is a lysosomal cysteine-proteinase that participates in the activation of serine proteinases in cells involved in immune and inflammatory processes, including phagocytosis of pathogens and local activation and deactivation of inflammatory factors (*Online Mendelian Inheritance in Man* [OMIM] number 602365). The SNP rs302652 at the *RAB38/CTSC* locus shows an eQTL in monocytes[38] associated with decreased expression of *RAB38*, possibly indicating that a decreased function of RAB38 might be the mechanism by which the association at this locus is mediated. Both *RAB38* and *CTSC* are implicated in lysosomal biology and an association with lysosomal and autophagic processes in FTD was previously suggested in two studies of *GRN*[51] and *TMEM106B*.[52] A possible role for autophagy has also been shown in Parkinson's disease.[53] Our findings will need to be replicated in other FTD cohorts in follow-up studies (eg, fine-mapping studies) to lend support to the inference that lysosomal biology and autophagy might be involved in the aetiology of FTD.[54]

The genetic association that we identified with the *HLA* locus lends support to the notion of a link between FTD and the immune system. Our mQTL data showed that risk at this locus is associated with *cis*-changes in methylation levels of *HLA-DRA* in the frontal cortex. *HLA* associations have been previously reported in Alzheimer's disease,[47] Parkinson's disease,[19,46] and multiple sclerosis.[44,45] Additionally, a general involvement of the innate and the adaptive immune responses has been suggested in the pathogenesis of neurodegenerative diseases,[55,56] lending support to the idea that the immune system plays an important part within the spectrum of neurological disorders (panel).

Future studies should aim to replicate our findings and elucidate the functional basis of FTD. Additionally, our data indicate that common pathways and processes might underlie different forms of neurodegenerative disorders, including Alzheimer's disease, Parkinson's disease, multiple sclerosis, and FTD. Exploring the possibility of developing therapeutic measures targeting general damage responses could hold promise—after replication and validation of our findings—for the development and implementation of treatment options for these neurological disorders, including FTD.

Contributors

JH, PM, ABS, MAN, RF, and JDR designed the study. JDR, RF and JH did the clinical quality checks. RF coordinated sample collection, received samples at UCL and TTUHSC, and did material quality control for discovery and replication phases. DGH received samples at NIH and coordinated material quality control at NIH. JDR, JBJK, CDS, PRS, WSB, JRH, GMH, OP, LB, ET, EH, IH, AR, MB BB, AP, LB, GB, RG, GF, DG, ES, CF, MS, JC, AL, RB, MLW, KN, CN, IRAM, G-YRH, DMAM, JG, CMM, JA, TDG, IGM, AJT, PP, EDH, EMW, AB, EJ, MCT, PP, CR, SO-C, EA, RP, JDS, PA, AK, IR, ER, LP, ER, PStG-H, ER, GR, FT, GG, JBR, JCMS, JU, JC, SM, AD, VMVD, MG, JQT, JvdZ, TVL, CVB, WD, MC, SFC, ILB, AB, DH, VG, MV, BN, SS, SB, IP, JEN, LEH, MR, BI, MM, GG, SP, WG, MNR, NCF, JDW, MGS, HM, PR, PH, JSS, AG, AR, SR, ACB, RM, FF, CC, LB, MA, MG, MEC, NS, RR, MB, DWD, JEP, NRGR, RCP, DK, KAJ, BFB, WWS, BLM, AMK, HR, JCvS, EGPD, HS, YALP, PS, GL, RC, VN, AAP, MF, AP, GM, PS, H-HC, CG, FP, AR, VD, FL, DK, LF, and SPB collected and characterised samples. MK was responsible for genotyping at ICH. JH, PM, ABS, and SPB obtained funding for this study. JH, PM, and ABS supervised the study. MAN did statistical and association analyses. RF, MAN, and JH analysed and interpreted the data. AR helped in the interpretation of the e/mQTL data. RF, MAN, JH, and PM wrote the first draft of the paper. All other co-authors participated in preparation of the paper by reading and commenting on drafts before submission.

Declaration of interests

CVB and MC are inventors on patent applications for *GRN* and *C9orf72*. PRS receives speaker fees from Janssen pharmaceutical. RR receives research support from the NIH (R01 NS080882, R01 NS065782, R01 AG026251, R01 NS076471, and P50 AG16574), the ALS Therapy Alliance, and the Consortium for Frontotemporal Degeneration Research, honoraria for lectures or educational activities not funded by industry. RR serves on the medical advisory board of the Association for Frontotemporal Degeneration and the board of directors of the International Society for Frontotemporal Dementia, and holds a patent on methods to screen for the hexanucleotide repeat expansion in the C9ORF72 gene. DWD is supported by NIH grants (P50 AG16574, P50 NS72187, P01 AG03949), the Mangurian Foundation, CurePSP, and the Robert E Jacoby Professorship for

Alzheimer's Research. NRGR is on the Scientific Advisory Board for Codman, TauRzx multicenter study, Consultation for CYTOX. RCP chairs a Data Monitoring Committee for Pfizer and Janssen Alzheimer Immunotherapy, and is a consultant for GE Healthcare and Elan Pharmaceuticals. RCP receives royalties from Oxford University Press for Mild Cognitive Impairment. DK has served on a data safety monitoring board for Lilly Pharmaceuticals, as a consultant to TauRx, was an investigator in clinical trials sponsored by Baxter, Elan Pharmaceuticals, and Forest Pharmaceuticals in the past 2 years and receives research support from the NIH. BFB has served as an investigator for clinical trials sponsored by Cephalon Inc, Allon Pharmaceuticals, and GE Healthcare. BFB receives royalties from the publication of a book entitled Behavioral Neurology Of Dementia (Cambridge Medicine, 2009). BFB has received honoraria from the American Academy of Neurology. BFB serves on the Scientific Advisory Board of the Tau Consortium. BFB receives research support from the National Institute on Aging (P50 AG016574, U01 AG006786, RO1 AG032306, RO1 AG041797) and the Mangurian Foundation. BLM is on the Board Membership of The Larry L Hillblom Foundation, The John Douglas French Foundation, The Tau Consortium, Sagol School of Neuroscience Tel Aviv University. BLM holds consultancy for Tau Rx lts—Chair, Scientific Advisory Board bvFTD Trial Allon Therapeutics—Steering Committee AL-108-231 Study, Bristol-Myers Squibb-Advisory Board, Progressive Supranuclear Palsy (PSP), Neurology Scientific Advisory Board Meeting Siemens Molecular Imaging, and Eli Lilly US Alzheimer's Disease Advisory Board, and receives royalties from Cambridge University Press Guilford Publications Inc, Neurocase. RF, DGH, MAN, JDR, AR, JBJK, CDS, WSB, GMH, JRH, OP, LB, ET, EH, IH, AR, MB, BB, AP, CC, NJC, LB, GB, RG, GF, DG, CF, MS, ES, JC, AL, RB, MLW, KN, CN, IRAM, G-YRH, DMAM, JG, CMM, JA, TDG, IGM, AJT, PP, EDH, EMW, AB, EJ, MCT, PP, CR, SO-C, EA, RP, JDS, PA, AK, IR, ER, LP, ER, PStG-H, GR, FT, GG, JBR, JCMS, JU, JC, SM, AD, VMVD, MG, JQT, JvdZ, WD, TVL, SFC, ILB, DH, VG, MV, AB, BN, SS, SB, IP, JEN, LEH, MR, MM, BI, GG, SP, WG, MNR, NCF, JDW, MGS, HRM, PR, PH, JSS, SR, AR, AG, ACB, RM, FF, CC, LB, MA, MG, MEC, NS, MB, KAJ, JEP, WWS, AMK, HR, JCvS, EGPD, HS, YALP, PS, GL, RC, VN, AAP, MF, AP, GM, PS, MK, H-HC, CG, FP, AR, VD, FL, DK, LF, SPB, JH, PM, and ABS declare no competing interests.

Acknowledgments

We received intramural funding from the National Institute of Neurological Disorders and Stroke (NINDS) and National Institute on Aging (NIA), the Wellcome/MRC Centre on Parkinson's disease, Alzheimer's Research UK (ARUK, Grant ARUK-PG2012-18), and by the office of the Dean of the School of Medicine, Department of Internal Medicine, at Texas Tech University Health Sciences Center. We thank Mike Hubank and Kerra Pearce at the Genomic core facility at the Institute of Child Health (ICH), UCL, for assisting RF in doing Illumina genotyping experiments (FTD-GWAS genotyping). The work done by the North American Brain Expression Consortium (NABEC) was supported in part by the Intramural Research Program of the National Institute on Aging, National Institutes of Health, part of the US Department of Health and Human Services (project number ZIA AG000932-04), and by a Research Grant from the Department of Defense (W81XWH-09-2-0128). Work done by the UK Brain Expression Consortium (UKBEC) was supported by the MRC through the MRC Sudden Death Brain Bank (CS), by a Project Grant (G0901254 to JH and MW), and by a Fellowship award (G0802462 to MR). DT was supported by the King Faisal Specialist Hospital and Research Centre, Saudi Arabia. Computing facilities used at King's College London were supported by the National Institute for Health Research (NIHR) Biomedical Research Centre based at Guy's and St Thomas' NHS Foundation Trust and King's College London. We thank AROS Applied Biotechnology AS company laboratories and Affymetrix for their valuable input. JBJK was supported by the National Health and Medical Resarch Council (NHMRC), Australia (project grants 510217 and 1005769). CDS was supported by NHMRC (project grants 630428 and 1005769). PRS was supported by NHMRC (project grants 510217 and 1005769) and acknowledges that DNA samples were prepared by Genetic Repositories Australia, supported by NHMRC Enabling Grant 401184. GMH was supported by NHMRC Research Fellowship 630434, Project Grant 1029538, and Program Grant 1037746. JRH was supported by the Australian Research Council Federation Fellowship, NHMRC Project Grant 1029538 and NHMRC Program Grant 1037746. OP was supported by NHMRC Career Development Fellowship 1022684, Project Grant 1003139. IH, AR, and MB acknowledge the patients and controls who participated in this project and the Trinitat Port-Carbó (deceased) and her extended family who are supporting Fundació ACE research programmes. CC was supported by Grant P30-NS069329-01 and acknowledges that the recruitment and clinical characterisation of research participants at Washington University were supported by NIH P50 AG05681, P01 AG03991, and P01 AG026276. LB and GB were supported by the Ricerca Corrente, Italian Ministry of Health. RG was supported by Fondazione CARIPLO 2009-2633, Ricerca Corrente, Italian Ministry of Health. GF was supported by Fondazione CARIPLO 2009–2633. ES was supported by the Italian Ministry of Health. CF was supported by Fondazione Cariplo. MS was supported from the Italian Ministry of Health (Ricerca Corrente). MLW was supported by Government funding of clinical research within NHS Sweden (ALF). KN was supported by Thure Carlsson Foundation. CN was supported by Swedish Alzheimer Fund. IRAM and GYRH were supported by CIHR (grant 74580) PARF (grant C06-01). JG was supported by the NINDS intramural research funds for FTD research. CMM was supported by Medical Research Council UK, Brains for Dementia Research, Alzheimer's Society, Alzheimer's Research UK, National Institutes for Health Research, Department of Health, and Yvonne Mairy Bequest, and acknowledges that tissue samples made available for this study were provided by the Newcastle Brain Tissue Resource, which was funded in part by grants G0400074 and G1100540 from the UK MRC, the Alzheimer's Research Trust and Alzheimer's Society through the Brains for Dementia Research Initiative and an NIHR Biomedical Research Centre Grant in Ageing and Health, and NIHR Biomedical Research Unit in Lewy Body Disorders. CMM was supported by the UK Department of Health and Medical Research Council and the Research was supported by the National Institute for Health Research Newcastle Biomedical Research Centre based at Newcastle Hospitals Foundation Trust and Newcastle University and acknowledges that the views expressed are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health. JA was supported by MRC, Dunhill Medical Trust, and Alzheimer's Research UK. TDG was supported by Wellcome Trust Senior Clinical Fellow. IGM was supported by NIHR Biomedical Research Centre and Unit on Ageing Grants and acknowledges the National Institute for Health Research Newcastle Biomedical Research Centre based at Newcastle Hospitals Foundation Trust and Newcastle University. The views expressed are those of the authors and not necessarily those of the NHS, the NIHR, or the Department of Health. AJT was supported by Medical Research Council, Alzheimer's Society, Alzheimer's Research UK, and the National Institutes for Health Research. EJ was supported by NIHR and Newcastle Biomedical Research Centre. PP, CR, SOC, and EA were supported partially by FIMA (Foundation for Applied Medical Research). PP acknowledges Manuel Seijo-Martínez (Department of Neurology, Hospital do Salnés, Pontevedra, Spain) and Ramon Rene, Jordi Gascon, and Jaume Campdelacreu (Department of Neurology, Hospital de Bellvitge, Barcelona, Spain) for providing FTD DNA samples. RP, JDS, PA, AK, and AD were supported by the German Federal Ministry of Education and Research (BMBF; grant number FKZ 01GI1007A—German FTLD consortium). IR was supported by Ministero dell'Istruzione, dell'Università e della Ricerca (MIUR) of Italy. PStG-H was supported by the Canadian Institutes of Health Research, Wellcome Trust, Ontario Research Fund. FT was supported by the Italian Ministry of Health (ricerca corrente) and MIUR grant RBAP11FRE9. GR and GG were supported by the Italian Ministry of Health (ricerca corrente). JBR was supported by Cambridge NIHR Biomedical Research Centre and Wellcome Trust (088324). JU, JC, and SM were supported by the MRC Prion Unit core funding and acknowledge MRC UK, UCLH Biomedical Research Centre, and Queen Square Dementia BRU. SM thanks John Beck, Tracy Campbell, Gary Adamson, Ron Druyeh, Jessica Lowe, and Mark Poulter. AD thanks Benedikt Bader, Manuela Neumann, Sigrun Roeber, Thomas Arzberger, and Hans Kretzschmar (deceased). VMVD and JQT were supported by grants AG032953, AG017586 and AG010124. MG was supported by Grants AG032953, AG017586, AG010124, and NS044266. VMVD thanks EunRan Suh for assistance with

sample handling and Elisabeth McCarty-Wood for help in selection of patients. JQT thanks Terry Schuck, John Robinson, and Kevin Raible for assistance with neuropathological assessment of patients. CVB and the Antwerp site were in part funded by the MetLife Foundation Award for Medical Research (to CVB), the Belgian Science Policy Office Interuniversity Attraction Poles programme; the Foundation for Alzheimer Research (SAO-FRA); the Medical Foundation Queen Elisabeth; the Flemish Government Methusalem Excellence award (to CVB); the Research Foundation Flanders (FWO); and the University of Antwerp Research Fund. JvdZ holds a postdoctoral fellowship of the FWO. CVB and the Antwerp site authors thanks neurologists S Engelborghs, P P De Deyn, A Sieben, and Rik Vandenberghe and neuropathologist J J Martin for the clinical and pathological diagnoses. Isabelle Leber and Alexis Brice were supported by the programme Investissements d'avenir ANR-10-IAIHU-06 and acknowledges the contribution of The French Research Network on FTLD/FTLD-ALS for the contribution in samples collection. BN, SS, SB, and IP were supported by Prin 2010-prot.2010PWNJXK; Cassa di Risparo di Firenze e Cassa di Risparmio di Pistoia e Pescia. JEN was supported by the Novo Nordisk Foundation, Denmark. MR was supported by the German National Genome Network (NGFN) and the German Ministry for Education and Research Grant Number 01GS0465. JDR, MNR, NCF, and JDW were supported by an MRC programme grant, the NIHR Queen Square Dementia Biomedical Research Unit and the Leonard Wolfson Experimental Neurology Centre. MGS was supported by MRC grant n G0301152, Cambridge Biomedical Research Centre, and thanks K Westmore for extracting DNA. HM was supported by the Motor Neuron Disease Association (Grant 6057). RR was supported by P50 AG016574, R01 NS080882, R01 NS065782, P50 NS72187, and the Consortium for Frontotemporal Dementia. DWD was supported by P50NS072187, P50AG016574, State of Florida Alzheimer Disease Initiative, and CurePSP Inc. NRGR, JEP, RCP, DK, and BFB were supported by P50 AG016574. KAJ was supported by R01 AG037491. WWS was supported by NIH AG023501, AG019724, Consortium for Frontotemporal Dementia Research. BLM was supported by P50AG023501, P01AG019724, Consortium for FTD Research. HR was supported by AG032306. JCvS was supported by Stichting Dioraphte Foundation (11 02 03 00), Nuts Ohra Foundation (0801-69), Hersenstichting Nederland (BG 2010-02), and Alzheimer Nederland. CG and HHC acknowledge families, patients, clinicians including Inger Nennesmo and Vesna Jelic, Laura Fratiglioni for control samples and Jenny Björkström, Håkan Thonberg, Charlotte Forsell, Anna-Karin Lindström, and Lena Lilius for sample handling. CG was supported by Swedish Brain Power (SBP), the Strategic Research Programme in Neuroscience at Karolinska Institutet (StratNeuro), the regional agreement on medical training and clinical research (ALF) between Stockholm County Council and Karolinska Institutet, Swedish Alzheimer Foundation, Swedish Research Council, Karolinska Institutet PhD-student funding, King Gustaf V, and Queen Victoria's Free Mason Foundation. FP, AR, VD, and FL acknowledge Labex DISTALZ. RF acknowledges the help and support of June Howard at the Texas Tech University Health Sciences Center Office of Sponsored Programs for tremendous help in managing Material Transfer Agreement at TTUHSC.

References

1 Neary D, Snowden JS, Gustafson L, et al. Frontotemporal lobar degeneration: a consensus on clinical diagnostic criteria. *Neurology* 1998; **51:** 1546–54.

2 Rabinovici GD, Miller BL. Frontotemporal lobar degeneration: epidemiology, pathophysiology, diagnosis and management. *CNS Drugs* 2010; **24:** 375–98.

3 Rohrer JD, Warren JD. Phenotypic signatures of genetic frontotemporal dementia. *Curr Opin Neurol* 2011; **24:** 542–49.

4 Rascovsky K, Hodges JR, Knopman D, et al. Sensitivity of revised diagnostic criteria for the behavioural variant of frontotemporal dementia. *Brain* 2011; **134:** 2456–77.

5 Gorno–Tempini ML, Hillis AE, Weintraub S, et al. Classification of primary progressive aphasia and its variants. *Neurology* 2011; **76:** 1006–14.

6 Mackenzie IR, Neumann M, Baborie A, et al. A harmonized classification system for FTLD–TDP pathology. *Acta Neuropathol* 2011; **122:** 111–13.

7 Halliday G, Bigio EH, Cairns NJ, Neumann M, Mackenzie IR, Mann DM. Mechanisms of disease in frontotemporal lobar degeneration: gain of function versus loss of function effects. *Acta Neuropathol* 2012; **124:** 373–82.

8 Hutton M, Lendon CL, Rizzu P, et al. Association of missense and 5′–splice–site mutations in tau with the inherited dementia FTDP–17. *Nature* 1998; **393:** 702–05.

9 Baker M, Mackenzie IR, Pickering-Brown SM, et al. Mutations in progranulin cause tau–negative frontotemporal dementia linked to chromosome 17. *Nature* 2006; **442:** 916–19.

10 Cruts M, Gijselinck I, van der Zee J, et al. Null mutations in progranulin cause ubiquitin-positive frontotemporal dementia linked to chromosome 17q21. *Nature* 2006; **442:** 920–4.

11 DeJesus–Hernandez M, Mackenzie IR, Boeve BF, et al. Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. *Neuron* 2011; **72:** 245–56.

12 Renton AE, Majounie E, Waite A, et al. A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. *Neuron* 2011; **72:** 257–68

13 Ferrari R, Thumma A, Momeni P. Molecular Genetics of Frontotemporal Dementia. In: eLS. John Wiley & Sons, Ltd: Chichester. 2013. DOI: 10.1002/9780470015902.a0024457.

14 van der Zee J, Gijselinck I, Dillen L , et al. A Pan-European Study of the C9orf72 Repeat Associated with FTLD: Geographic Prevalence, Genomic Instability, and Intermediate Repeats. *Hum Mutat* 2013; **34:** 363–73.

15 Rohrer JD, Rosen HJ. Neuroimaging in frontotemporal dementia. *Int Rev Psychiatry* 2013; **25:** 221–29.

16 Ling SC, Polymenidou M, Cleveland DW. Converging Mechanisms in ALS and FTD: Disrupted RNA and Protein Homeostasis. *Neuron* 2013; **79:** 416–38.

17 Van Deerlin VM, Sleiman PM, Martinez–Lage M, et al. Common variants at 7p21 are associated with frontotemporal lobar degeneration with TDP-43 inclusions. *Nat Genet* 2010; **42:** 234–39.

18 Strong MJ, Grace GM, Freedman M, et al. Consensus criteria for the diagnosis of frontotemporal cognitive and behavioural syndromes in amyotrophic lateral sclerosis. *Amyotroph Lateral Scler* 2009; **10:** 131–46.

19 International Parkinson Disease Genomics Consortium, Nalls MA, Plagnol V, et al. Imputation of sequence variants for identification of genetic risks for Parkinson's disease: a meta-analysis of genome-wide association studies. *Lancet* 2011; **377:** 641–9.

20 1000 Genomes Project Consortium, Abecasis GR, Auton A, et al. An integrated map of genetic variation from 1092 human genomes. *Nature* 2012; **491:** 56–65.

21 International HapMap 3 Consortium, Altshuler DM, Gibbs RA,, et al. Integrating common and rare genetic variation in diverse human populations. *Nature* 2010; **467:** 52–58.

22 Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal components analysis corrects for stratification in genome-wide association studies. *Nature Gen* 2006; **38:** 904–09.

23 Grove ML, Yu B, Cochran BJ, et al. Best Practices and Joint Calling of the HumanExome BeadChip: The CHARGE Consortium. *PLoS One* 2013; **8:** e68095.

24 Millar T, Walker R, Arango JC, et al. Tissue and organ donation for research in forensic pathology: the MRC Sudden Death Brainand Tissue Bank. *J Pathol* 2007; **213:** 369–75.

25 Beach TG, Sue LI, Walker DG, et al. The Sun Health Research Institute Brain Donation Program: description and experience, 1987–2007. *Cell Tissue Bank* 2008; **9:** 229–45.

26 Hawrylycz MJ, Lein ES, Guillozet–Bongaarts AL, et al. An anatomically comprehensive atlas of the adult human brain transcriptome. *Nature* 2012; **489:** 391–99.

27 Kang HJ, Kawasawa YI, Cheng F, et al. Spatio-temporal transcriptome of the human brain. *Nature* 2011; **478:** 483–89.

28 Roth RB, Hevezi P, Lee J, et al. Gene expression analyses reveal molecular relationships among 20 regions of the human CNS. *Neurogenetics* 2006; **7:** 67–80.

29 Trabzuni D, Ryten M, Walker R, et al. Quality control parameters on a large dataset of regionally dissected human control brains for whole genome expression studies. *J Neurochem* 2011; **119:** 275–82.

30 Irizarry RA, Hobbs B, Collin F, et al. Exploration, normalization, and summaries of high density oligonucleotide array probe level data. *Biostatistics* 2003; **4:** 249–64.

31 Ramasamy A, Trabzuni D, Gibbs JR, et al. Resolving the polymorphism-in-probe problem is critical for correct interpretation of expression QTL studies. *Nucleic Acids Res* 2013; **41:** e88.

32 International Parkinson's Disease Genomics Consortium (IPDGC); Wellcome Trust Case Control Consortium 2 (WTCCC2). A two-stage meta-analysis identifies several new loci for Parkinson's disease. *PLoS Genet* 2011; **7:** e1002142.

33 Li Y, Willer C, Sanna S, Abecasis G. Genotype imputation. *Annu Rev Genomics Hum Genet* 2009; **10:** 387–406.

34 Li Y, Willer CJ, Ding J, Scheet P, Abecasis GR. MaCH: using sequence and genotype data to estimate haplotypes and unobserved genotypes. *Genet Epidemiol* 2010; **34:** 816–34.

35 Gibbs JR, van der Brug MP, Hernandez DG, et al. Abundant quantitative trait loci exist for DNA methylation and gene expression in human brain. *PLoS Genet* 2010; **6:** e1000952.

36 Barbosa-Morais NL, Dunning MJ, Samarajiwa SA, et al. A re-annotation pipeline for Illumina BeadArrays: improving the interpretation of gene expression data. *Nucleic Acids Res* 2010; **38:** e1.7.

37 Shabalin AA. Matrix eQTL: Ultra fast eQTL analysis via large matrix operations. *Bioinformatics* 2012; **28:** 1353–58.

38 Zeller T, Wild P, Szymczak S, et al. Genetics and beyond the transcriptome of human monocytes and disease susceptibility. *PLoS One* 2010; **5:** e10693.

39 van Es MA, Veldink JH, Saris CG, et al. Genome-wide association study identifies 19p13.3 (UNC13A) and 9p21.2 as susceptibility loci for sporadic amyotrophic lateral sclerosis. *Nat Genet* 2009; **41:** 1083–87.

40 Höglinger GU, Melhem NM, Dickson DW, et al. Identification of common variants influencing risk of the tauopathy progressive supranuclear palsy. *Nat Genet* 2011; **43:** 699–705.

41 Seshadri S, Fitzpatrick AL, Ikram MA, et al. Genome-wide analysis of genetic loci associated with Alzheimer disease. *JAMA* 2010; **303:** 1832–40.

42 van der Zee J, Sleegers K, Van Broeckhoven C. Invited article: the Alzheimer disease-frontotemporal lobar degeneration spectrum. *Neurology* 2008; **71:** 1191–97.

43 Baranzini SE, Wang J, Gibson RA, et al. Genome wide association analysis of susceptibility and clinical phenotype in multiple sclerosis. *Hum Mol Genet* 2009; **18:** 767–78.

44 International Multiple Sclerosis Genetics Consortium, Hafler DA, Compston A, et al. Risk alleles for multiple sclerosis identified by a genomewide study. *N Engl J Med* 2007; **357:** 851–62.

45 Mero IL, Gustavsen MW, Sæther HS, et al. Oligoclonal band status in Scandinavian multiple sclerosis patients is associated with specific genetic risk alleles. *PLoS One* 2013; **8:** e58352.

46 Hamza TH, Zabetian CP, Tenesa A, et al. Common genetic variation in the HLA region is associated with late-onset sporadic Parkinson's disease. *Nat Genet* 2010; **42:** 781–85.

47 Lambert JC, Ibrahim-Verbaas CA, Harold D, et al. Meta-analysis of 74046 individuals identifies 11 new susceptibility loci for Alzheimer's disease. *Nat Genet* 2013; **45:** 1452–458.

48 Jäger D, Stockert E, Jäger E, et al. Serological cloning of a melanocyte rab guanosine 5-prime-triphosphate-binding protein and a chromosome condensation protein from a melanoma complementary DNA library. *Cancer Res* 2000; **60:** 3584–91.

49 Bultema JJ, Ambrosio AL, Burek CL, Di Pietro SM. BLOC-2, AP-3, and AP-1 proteins function in concert with Rab38 and Rab32 proteins to mediate protein trafficking to lysosome–related organelles. *J Biol Chem* 2012; **287:** 19550–63.

50 Seto S, Tsujimura K, Koide Y. Rab GTPAses regulating phagosome maturation are differentially recruited to mycobacterial phagosomes. *Traffic* 2011; **12:** 407–20.

51 Hu F, Padukkavidana T, Vægter CB, et al. Sortilin–mediated endocytosis determines levels of the frontotemporal dementia protein, progranulin. *Neuron* 2010; **68:** 654–67.

52 Brady OA, Zheng Y, Murphy K, Huang M, Hu F. The frontotemporal lobar degeneration risk factor, TMEM106B, regulates lysosomal morphology and function. *Hum Mol Genet* 2013; **22:** 685–95.

53 Westbroek W, Gustafson AM, Sidransky E. Exploring the link between glucocerebrosidase mutations and parkinsonism. *Trends Mol Med* 2011; **17:** 485–93.

54 Hardy J, Rogaeva E. Motor neuron disease and frontotemporal dementia: sometimes related, sometimes not. *Exp Neurol* 2013; published online Nov 15. DOI:10.1016/j.expneurol.2013.11.006.

55 Amor S, Woodroofe MN. Innate and adaptive immune responses in neurodegeneration and repair. *Immunology* 2014; **141:** 287–91.

56 Träger U, Tabrizi SJ. Peripheral inflammation in neurodegeneration. *J Mol Med* 2013; **91:** 673–81.

Acta Neuropathol
DOI 10.1007/s00401-014-1298-7

ORIGINAL PAPER

Rare mutations in *SQSTM1* modify susceptibility to frontotemporal lobar degeneration

Julie van der Zee · Tim Van Langenhove · Gabor G. Kovacs · Lubina Dillen · William Deschamps · Sebastiaan Engelborghs · Radoslav Matěj · Mathieu Vandenbulcke · Anne Sieben · Bart Dermaut · Katrien Smets · Philip Van Damme · Céline Merlin · Annelies Laureys · Marleen Van Den Broeck · Maria Mattheijssens · Karin Peeters · Luisa Benussi · Giuliano Binetti · Roberta Ghidoni · Barbara Borroni · Alessandro Padovani · Silvana Archetti · Pau Pastor · Cristina Razquin · Sara Ortega-Cubero · Isabel Hernández · Mercè Boada · Agustín Ruiz · Alexandre de Mendonça · Gabriel Miltenberger-Miltényi · Frederico Simões do Couto · Sandro Sorbi · Benedetta Nacmias · Silvia Bagnoli · Caroline Graff · Huei-Hsin Chiang · Håkan Thonberg · Robert Perneczky · Janine Diehl-Schmid · Panagiotis Alexopoulos · Giovanni B. Frisoni · Christian Bonvicini · Matthis Synofzik · Walter Maetzler · Jennifer Müller vom Hagen · Ludger Schöls · Tobias B. Haack · Tim M. Strom · Holger Prokisch · Oriol Dols-Icardo · Jordi Clarimón · Alberto Lleó · Isabel Santana · Maria Rosário Almeida · Beatriz Santiago · Michael T. Heneka · Frank Jessen · Alfredo Ramirez · Raquel Sanchez-Valle · Albert Llado · Ellen Gelpi · Stayko Sarafov · Ivailo Tournev · Albena Jordanova · Eva Parobkova · Gian Maria Fabrizi · Silvia Testi · Eric Salmon · Thomas Ströbel · Patrick Santens · Wim Robberecht · Peter De Jonghe · Jean-Jacques Martin · Patrick Cras · Rik Vandenberghe · Peter Paul De Deyn · Marc Cruts · Kristel Sleegers · Christine Van Broeckhoven

Received: 10 February 2014 / Revised: 12 May 2014 / Accepted: 20 May 2014

Abstract Mutations in the gene coding for Sequestosome 1 (SQSTM1) have been genetically associated with amyotrophic lateral sclerosis (ALS) and Paget disease of bone. In the present study, we analyzed the *SQSTM1* coding sequence for mutations in an extended cohort of 1,808 patients with frontotemporal lobar degeneration (FTLD), ascertained within the European Early-Onset Dementia consortium. As control dataset, we sequenced 1,625 European control individuals and analyzed whole-exome sequence data of 2,274 German individuals (total $n = 3{,}899$). Association of rare *SQSTM1* mutations was calculated in a meta-analysis of 4,332 FTLD and 10,240 control alleles. We identified 25 coding variants in FTLD patients of which 10 have

On behalf of the BELNEU consortium and of the EU EOD consortium are given in Appendix.

The members of BELNEU consortium and EU EOD consortium are given in Appendix.

Electronic supplementary material The online version of this article (doi:10.1007/s00401-014-1298-7) contains supplementary material, which is available to authorized users.

J. van der Zee · T. Van Langenhove · L. Dillen · W. Deschamps · A. Sieben · K. Smets · C. Merlin · A. Laureys · M. Van Den Broeck · M. Mattheijssens · K. Peeters · A. Jordanova · P. De Jonghe · J.-J. Martin · M. Cruts · K. Sleegers · C. Van Broeckhoven
Department of Molecular Genetics, VIB, Antwerp, Belgium

J. van der Zee · T. Van Langenhove · L. Dillen · W. Deschamps · S. Engelborghs · A. Sieben · K. Smets · C. Merlin · A. Laureys · M. Van Den Broeck · M. Mattheijssens · K. Peeters · A. Jordanova · P. De Jonghe · J.-J. Martin · P. Cras · P. P. De Deyn · M. Cruts · K. Sleegers · C. Van Broeckhoven (✉)
Institute Born-Bunge, University of Antwerp, Antwerp, Belgium
e-mail: christine.vanbroeckhoven@molgen.vib-ua.be

T. Van Langenhove · K. Smets · P. De Jonghe · P. Cras
Department of Neurology, Antwerp University Hospital, Edegem, Belgium

G. G. Kovacs · T. Ströbel
Institute of Neurology, Neurodegenerative Diseases Group, Medical University of Vienna, Vienna, Austria

S. Engelborghs · P. P. De Deyn
Department of Neurology and Memory Clinic, Hospital Network Antwerp Middelheim and Hoge Beuken, Antwerp, Belgium

R. Matěj · E. Parobkova
Department of Pathology and Molecular Medicine, Thomayer Hospital, Prague, Czech Republic

not been described. Fifteen mutations were absent in the control individuals (carrier frequency <0.00026) whilst the others were rare in both patients and control individuals. When pooling all variants with a minor allele frequency <0.01, an overall frequency of 3.2 % was calculated in patients. Rare variant association analysis between patients and controls showed no difference over the whole protein, but suggested that rare mutations clustering in the UBA domain of *SQSTM1* may influence disease susceptibility by doubling the risk for FTLD (RR = 2.18 [95 % CI 1.24–3.85]; corrected p value = 0.042). Detailed histopathology demonstrated that mutations in *SQSTM1* associate with widespread neuronal and glial phospho-TDP-43 pathology. With this study, we provide further evidence for a putative role of rare mutations in *SQSTM1* in the genetic etiology of FTLD and showed that, comparable to other FTLD/ALS genes, *SQSTM1* mutations are associated with TDP-43 pathology.

Keywords Sequestosome 1 · *SQSTM1* · p62 · FTLD · ALS · Rare variants

Introduction

Frontotemporal lobar degeneration (FTLD) represents a heterogeneous group of progressive neurodegenerative dementias, caused by local atrophy of frontal and/or temporal lobes. It is one of the most common forms of early-onset dementia (EOD), with the majority of FTLD patients developing disease between 45 and 65 years. About 15 % of FTLD patients present with a motor neuron disease (MND) syndrome, most commonly amyotrophic lateral sclerosis (ALS). Like FTLD, ALS is a neurodegenerative disorder in which loss of motor neurons leads to progressive weakness of the voluntary muscles. FTLD and ALS show important genetic overlap with mutations identified in the same genes, e.g., the common G_4C_2 repeat expansion in the chromosome 9 open reading frame 72 gene (*C9orf72*) and less frequently, mutations in the valosin containing protein (*VCP),* fused in sarcoma (*FUS)*, TAR DNA-binding protein (*TARDBP)* and ubiquilin 2 (*UBQLN2*) genes [5, 8, 30, 31, 37].

Sequencing of the gene coding for *sequestosome 1* (*SQSTM1*) in ALS patients identified several rare mutations

R. Matěj
Department of Neurology, First Medical Faculty, Center of Clinical Neurosciences, Charles University in Prague, Prague, Czech Republic

M. Vandenbulcke
Brain and Emotion Laboratory, Department of Psychiatry, University of Leuven, Louvain, Belgium

M. Vandenbulcke · R. Vandenberghe
Old Age Psychiatry, University Hospitals Leuven and Department of Neurosciences, University of Leuven, Louvain, Belgium

A. Sieben · B. Dermaut · P. Santens
Department of Neurology, University Hospital Ghent, Ghent, Belgium

B. Dermaut
Center for Medical Genetics, University Hospital Ghent, Ghent, Belgium

B. Dermaut
Inserm U744, Institut Pasteur de Lille, Université de Lille Nord de France, Lille, France

P. Van Damme · W. Robberecht
Department of Neurology, University Hospitals Leuven and University of Leuven, Louvain, Belgium

P. Van Damme · W. Robberecht
Laboratory for Neurobiology, Vesalius Research Center, VIB, Louvain, Belgium

L. Benussi · G. Binetti
NeuroBioGen Lab-Memory Clinic, IRCCS Istituto Centro San Giovanni di Dio Fatebenefratelli, Brescia, Italy

R. Ghidoni
Proteomics Unit, IRCCS Istituto Centro San Giovanni di Dio Fatebenefratelli, Brescia, Italy

B. Borroni · A. Padovani
Neurology Unit, University of Brescia, Brescia, Italy

S. Archetti
III Laboratory of Analysis, Brescia Hospital, Brescia, Italy

P. Pastor · C. Razquin · S. Ortega-Cubero
Neurogenetics Laboratory, Division of Neurosciences, Center for Applied Medical Research, Universidad de Navarra, Pamplona, Spain

P. Pastor · S. Ortega-Cubero
Department of Neurology, Clínica Universidad de Navarra, University of Navarra School of Medicine, Pamplona, Spain

P. Pastor · S. Ortega-Cubero
Centro de Investigación Biomédica en Red de Enfermedades Neurodegenerativas, Instituto de Salud Carlos III, Madrid, Spain

I. Hernández · M. Boada · A. Ruiz
Memory Clinic of Fundació ACE, Institut Català de Neurociències Aplicades, Barcelona, Spain

A. de Mendonça · G. Miltenberger-Miltényi · F. S. do Couto
Faculty of Medicine and Institute of Molecular Medicine, University of Lisbon, Lisbon, Portugal

F. S. do Couto
Hospital Santa Maria, Lisbon, Portugal

[7]. Some of these mutations had been associated with Paget disease of bone (PDB [9, 14, 16]), a localized chronic bone disorder characterized by abnormalities of bone architecture and marrow fibrosis, resulting in an osteodystrophia deformans. Of interest, FTLD, PDB and inclusion body myopathy (IBM) had previously been genetically linked by mutations in *VCP* [13, 36, 38]. Recently, rare mutations in *SQSTM1* were also reported in FTLD patients [17, 28]. The observation of rare mutations (1–3 %) in both FTLD and ALS patients suggested an involvement of the protein SQSTM1, also known as p62, in these pathologies possibly through a common disease pathomechanism. The p62 protein is a stress-responsive ubiquitin-binding protein shown to have a role in degradation of polyubiquitinated proteins via the proteasome pathway or autophagic processes [26]. It is present in neuronal and glial ubiquitin-positive inclusions in different tauopathies and synucleinopathies, including Alzheimer disease, FTLD, dementia with Lewy bodies, Parkinson disease, Huntington disease and multiple system atrophy [15, 20, 23]. Also in FTLD, with or without ALS, p62 co-localizes with TDP-43 and FUS in brain and/or spinal cord [2, 6, 32]. Recently, p62 positive but TDP-43 negative immunoreactivity, extending to the pyramidal cell layer of the hippocampus, basal ganglia and cerebellum, has been recognized as a distinctive feature of *C9orf72*-associated FTLD and ALS [1, 34]. Aggregating dipeptide repeats (DPRs), translated from the expanded GGGGCC repeat, were identified as the main component of these inclusions [21, 22]. In the present study, we aimed at determining the genetic contribution of mutations in *SQSTM1* to the etiology of FTLD. Hereto, we analyzed a large study population of 1,808 FTLD patients and compared mutation data to a set of 3,899 European control individuals, as well as 395 European ALS patients.

Materials and methods

The patient and control cohorts under investigation were ascertained through the European Early-Onset Dementia (EU EOD) consortium (Supplementary table 1) [35]. For the present study, DNA and medical/demographic

S. Sorbi · B. Nacmias · S. Bagnoli
Department of Neurosciences, Psychology, Drug Research and Child Health (NEUROFARBA), University of Florence, Florence, Italy

C. Graff · H.-H. Chiang
Karolinska Institutet, Department of Neurobiology, Care Sciences and Society (NVS), KI-Alzheimer Disease Research Center, Stockholm, Sweden

C. Graff · H.-H. Chiang · H. Thonberg
Genetics Unit, Department of Geriatric Medicine, Karolinska University Hospital, Stockholm, Sweden

R. Perneczky
Neuroepidemiology and Ageing Research Unit, School of Public Health, Faculty of Medicine, The Imperial College of Science, Technology and Medicine, London W6 8RP, UK

R. Perneczky
West London Cognitive Disorders Treatment and Research Unit, West London Mental Health Trust, London TW8 8DS, UK

R. Perneczky · J. Diehl-Schmid · P. Alexopoulos
Department of Psychiatry and Psychotherapy, Technische Universität München, 81675 Munich, Germany

G. B. Frisoni
Hôpitaux Universitaires de Genève et Université de Genève, Geneva, Switzerland

G. B. Frisoni · C. Bonvicini
IRCCS Fatebenefratelli, Brescia, Italy

M. Synofzik · W. Maetzler · J. M. vom Hagen · L. Schöls
Department of Neurodegeneration, Hertie Institute for Clinical Brain Research and Centre of Neurology, Tübingen, Germany

M. Synofzik · W. Maetzler · J. M. vom Hagen · L. Schöls
German Research Center for Neurodegenerative Diseases (DZNE), Tübingen, Germany

T. B. Haack · T. M. Strom · H. Prokisch
Institute of Human Genetics, Technische Universität München, 81675 Munich, Germany

T. B. Haack · T. M. Strom · H. Prokisch
Institute of Human Genetics, Helmholtz Zentrum München, 85764 Neuherberg, Germany

O. Dols-Icardo · J. Clarimón · A. Lleó
Department of Neurology, IIB Sant Pau, Hospital de la Santa Creu i Sant Pau, Universitat Autònoma de Barcelona, Barcelona, Spain

O. Dols-Icardo · J. Clarimón · A. Lleó
Center for Networker Biomedical Research in Neurodegenerative Diseases (CIBERNED), Madrid, Spain

I. Santana · B. Santiago
Neurology Department, Centro Hospitalar Universitário de Coimbra, Coimbra, Portugal

I. Santana
Faculty of Medicine, University of Coimbra, Coimbra, Portugal

M. R. Almeida
Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal

M. T. Heneka
Clinical Neuroscience Unit, Department of Neurology, University of Bonn, Bonn, Germany

information on 1,808 FTLD patients, originating from Belgium, Italy, Germany, Spain, Portugal, Sweden, Czech Republic, Bulgaria and Austria, was contributed by members of the consortium. From this patient cohort, 1,706 patients were clinically diagnosed with FTLD and 102 with concomitant FTLD and ALS (FTLD-ALS) (for the remainder of the manuscript, the FTLD group will refer to the 1,706 FTLD patients plus the 102 FTLD-ALS patients). The research question of this study was whether genetic variations in *SQSTM1* affect FTLD risk. Yet, because of the close relationship with FTLD, the contributed patient cohorts also included a number of ALS patients ($n = 395$). Patients were evaluated and diagnosed with FTLD according to the Lund and Manchester group criteria [25] and for ALS according to the revised El Escorial criteria [3]. Clinical diagnoses of behavioral variant frontotemporal dementia (bvFTD) were based on the international consensus criteria by Rascovsky et al. [27] and of progressive supranuclear palsy (PSP) on the National Institute of Neurological Disorders and the Society for PSP criteria [18]. Neuropathological examination was performed in 105 autopsied patients, including 67 with FTLD-TDP, 2 FTLD-UPS, 3 FTLD-tau, 1 FTLD-ni, 4 FTLD unspecified, 21 ALS-TDP and 7 ALS unspecified. Genetic mutation profiling of FTLD- and ALS-associated genes was performed for *C9orf72* ($n = 2{,}055$), GRN ($n = 1{,}024$), MAPT ($n = 854$), VCP ($n = 159$), CHMP2B ($n = 153$), TARDBP ($n = 272$) and FUS ($n = 184$) and revealed 150 C9orf72 repeat expansion mutations, 24 GRN, 5 MAPT, 2 VCP, 1 CHMP2B, and 2 FUS mutations. A positive family history was defined for index patients with first- or second-degree relatives with symptoms of dementia or MND. Patients were classified as sporadic when no other affected family members were reported. Patients from whom no information on family history could be obtained were classified as 'family history undocumented'. As control group, we sequenced 1,625 age- and origin-matched Western-European individuals with no personal or family history of neurodegenerative or psychiatric diseases and a Mini Mental State Examination (MMSE) score >26. We further analyzed whole-exome sequencing (WES) data of 2,274 German non-demented individuals [10, 40]. Together, 3,899 control persons were investigated for coding variants in *SQSTM1*.

For all participants, informed consent for participation in the genetic studies was obtained according to sampling protocols that were approved by the Ethics Committee of the respective hospitals. The protocols for the genetic studies were approved by the Ethics Committee of the University of Antwerp, Belgium.

Sample quality control

30 µl at 20 ng/µl of genomic DNA (gDNA) was requested, and concentration and purity were checked spectrophotometrically using the Trinean DropSense96 UV/VIS droplet reader for all consortium samples. Gender and DNA fingerprint were determined for all DNA samples using an in-house developed multiplex polymerase-chain reaction

M. T. Heneka · F. Jessen
German Center for Neurodegenerative Diseases (DZNE), University of Bonn, Bonn, Germany

F. Jessen · A. Ramirez
Department of Psychiatry and Psychotherapy, University of Bonn, Bonn, Germany

A. Ramirez
Institute of Human Genetics, University of Bonn, Bonn, Germany

R. Sanchez-Valle · A. Llado
Alzheimer's Disease and Other Cognitive Disorders Unit, Neurology Department, Hospital Clínic, IDIBAPS, Barcelona, Spain

E. Gelpi
Neurological Tissue Bank of the Biobanc-Hospital Clinic-Institut d'Investigacions Biomediques August Pi i Sunyer (IDIBAPS), Barcelona, Spain

S. Sarafov · I. Tournev
Department of Neurology, Medical University Sofia, Sofia, Bulgaria

I. Tournev
Department of Cognitive Science and Psychology, New Bulgarian University, Sofia, Bulgaria

A. Jordanova
Department of Biochemistry, Molecular Medicine Center, Medical University, Sofia, Sofia, Bulgaria

G. M. Fabrizi · S. Testi
Department of Neurological and Movement Sciences, University of Verona, Verona, Italy

E. Salmon
Cyclotron Research Centre, University of Liege and Memory Clinic, CHU Liege, Liege, Belgium

R. Vandenberghe
Laboratory for Cognitive Neurology, Department of Neurology, University of Leuven and University Hospitals Leuven Gasthuisberg, Louvain, Belgium

P. P. De Deyn
Department of Neurology and Alzheimer Research Center, University of Groningen and University Medical Center Groningen, Groningen, The Netherlands

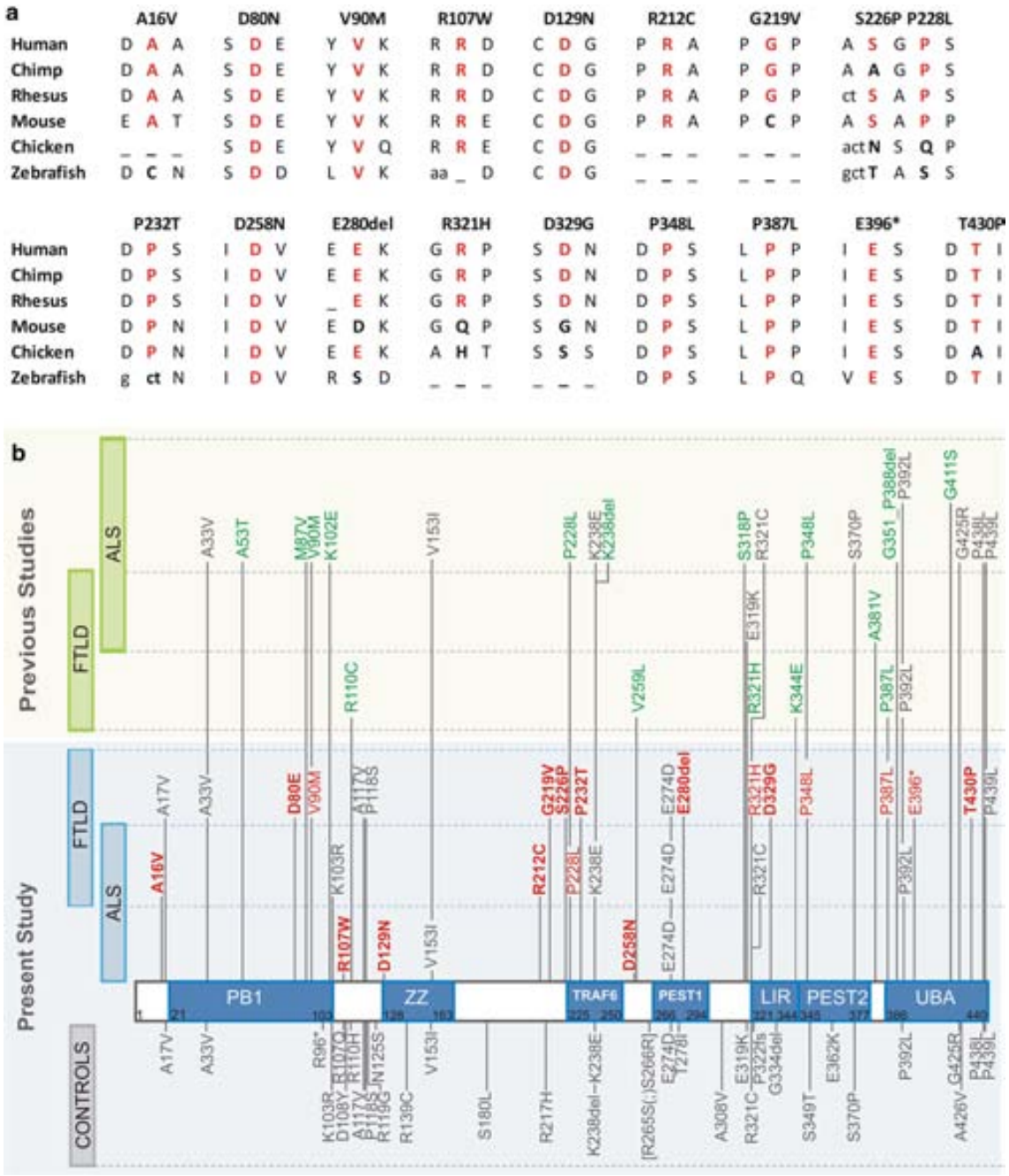


Fig. 1 *SQSTM1* mutations identified in FTLD and ALS patient cohorts ascertained with the European EOD consortium. **a** Sequence alignment for patient-specific mutations showing evolutionary conservation across species. **b** In the *blue panel*, *SQSTM1* mutations identified in the present study in patients (*top*) and control individuals (*bottom*) are presented on the primary structure if the p62 protein indicating known functional domains. Mutations absent from tested and published controls are in *red*. Mutations not previously associated with FTLD, ALS, or PDB are in *red* and *bold*. In the *green panel*, *SQSTM1* mutations reported in previous studies are given [7, 11, 17, 28, 29, 33]. Mutations absent from tested and published controls are in *green*. Functional domains according to [11]: PB1 = Phox and Bem1p domain; ZZ = zinc finger motif; TRAF6 = TNF receptor–associated factor 6; LIR = LC3 interaction region; PEST1 = proline (P), glutamic acid (E), serine (S), and threonine (T) domain 1; PEST2 = PEST domain 2; UBA = ubiquitin-associated domain

(PCR) genomic DNA Fingerprint panel comprising 13 short tandem repeat (STR) markers distributed over multiple autosomal loci—D20S480, D22S1174, D3S1287, D3S1744, D3S1764, D7S672, D7S2426, D8S1746, D14S1005, D20S866, D10S1237, D20S912, D6S965—and two sex chromosome markers—DXS1187, chrom Y: 2655362–2655672—to enable fast and accurate sample identification and gender determination in a single PCR.

Table 1 *SQSTM1* mutations present only in patients and associated clinical phenotypes

Mutation	Functional domain	Origin	Gender	Clinical diagnosis	Sub-diagnosis[b]	Family history	Age at onset (years)	Age at death (years)
FTLD								
p.Ala16Val[a]		Italian	M	FTLD-ALS		U	71	74
p.Asp80Glu[a]	PB1	Italian	F	FTLD	bvFTD	F	71	85
p.Val90Met	PB1	Portuguese	F	FTLD	bvFTD	U	41	
p.Arg212Cys[a]		Austrian	M	FTLD-ALS		F	63	66
p.Gly219Val[a]		Portuguese	M	FTLD	bvFTD	F	52	
p.Ser226Pro[a]	TRAF6	Spanish	M	FTLD	bvFTD	S	61	
p.Pro228Leu	TRAF6	German	M	FTLD	bvFTD	S	57	
p.Pro232Thr[a]	TRAF6	Portuguese	F	FTLD[c]	bvFTD	F	55	68
p.Glu280del[a]	PEST1	Italian	F	FTLD	PSP	F	73	
p.Arg321His	LIR	Italian	F	FTLD		U	68	
p.Asp329Gly[a]	LIR	Spanish	M	FTLD	bvFTD	U	78	84
p.Pro348Leu	PEST2	Italian	M	FTLD	PNFA	F	74	
p.Pro387Leu	UBA	Italian	F	FTLD	PNFA	F	65	
p.Pro387Leu	UBA	Italian	M	FTLD	bvFTD	S	66	
p.(Glu396*)	UBA	Czech	M	FTLD	bvFTD	S	43	47
p.Thr430Pro[a]	UBA	Portuguese	M	FTLD	bvFTD	S	58	63
ALS								
p.Arg107Trp[a]		Spanish	F	ALS	MND	S	58	62
p.Asp129Asn[a]	ZZ	Flemish	M	ALS		S	62	
p.Asp258Asn[a]		German	F	ALS		F	52	62

Functional domains according to [11] (Fig. 1b)

bvFTD behavioral variant frontotemporal dementia, *MND* motor neuron disease, *PSP* progressive supranuclear palsy, *PNFA* progressive non-fluent aphasia, *F* familial, *S* sporadic, *U* family history undocumented

[a] Indicates variants not previously associated with ALS, FTLD or PDB [7, 11, 17, 28, 29, 33]. For a complete description of SQSTM1 mutations, see Supplementary table 2

[b] Clinical subdiagnosis is given where documented

[c] After revision of the medical records of the mutation carriers, a diagnosis of possible PDB was made in hindsight in this patient

Table 2 *SQSTM1* mutations present in patients and control individuals

Mutation	Functional domain	FTLD $n = 1{,}808$	ALS $n = 395$	Controls $n = 3{,}899$
p.Ala17Val[a]		1		1
p.Ala33Val	PB1	1		1
p.Lys103Arg	PB1	1		2
p.Ala117Val		1		3
p.Pro118Ser		1		2
p.Val153Ile	ZZ		1	3
p.Lys238Glu	TRAF6	17	6	14
p.Glu274Asp	PEST1	109	22	79
p.Arg321Cys	LIR	3	1	1
p.Pro392Leu	UBA	15	3	11
p.Pro439Leu	UBA	2		2

Functional domains according to [11] (Fig. 1b)

[a] Indicates variants not previously associated with ALS, FTLD or PDB [7, 11, 17, 28, 29, 33]. For a complete description of the SQSTM1 mutations, see Supplementary table 2

After selective amplification of 20 ng gDNA under empirically defined reaction conditions, amplification products were size separated on an ABI 3730 automatic sequencer (Applied Biosystems) using GeneScan-600 LIZ (Applied Biosystems) as internal size standard and genotypes were assigned using in-house developed TracI genotyping software (http://www.vibgeneticservicefacility.be). Duplicate samples, gender mismatches and failed samples due to low

Table 3 Descriptives of the *SQSTM1* variants found in control individuals only

On cDNA level	Exon	On protein level	Functional domain	dbSNP
NM_003900.4:c.286C>T	Exon 2	NP_003891.1:p.(Arg96*)	PB1	
NM_003900.4:c.308A>G	Exon 3	NP_003891.1:p.Arg107Gln		
NM_003900.4:c.322G>T	Exon 3	NP_003891.1:p.Asp108Tyr		
NM_003900.4:c.329G>A	Exon 3	NP_003891.1:p.Arg110His		
NM_003900.4:c.355C>G	Exon 3	NP_003891.1:p.Arg119Gly		
NM_003900.4:c.374A>G	Exon 3	NP_003891.1:p.Asn125Ser		
NM_003900.4:c.415C>T	Exon 3	NP_003891.1:p.Arg139Cys	ZZ	
NM_003900.4:c.539C>T	Exon 4	NP_003891.1:p.Ser180Leu		
NM_003900.4:c.650G>A	Exon 4	NP_003891.1:p.Arg217His		
NM_003900.4:c.711_713delGAA	Exon 5	NP_003891.1:p.lys238del	TRAF6	
NM_003900.4:c.795_796delinsTT	Exon 6	NP_003891.1:p.[Arg265Ser(;) Ser266Arg]	PEST1	
NM_003900.4:c.833C>T	Exon 6	NP_003891.1:p.Thr278Ile	PEST1	rs200445838
NM_003900.4:c.923C>T	Exon 6	NP_003891.1:p.Ala308Val		
NM_003900.4:c.955G>A	Exon 6	NP_003891.1:p.Glu319lys		rs61748794
NM_003900.4:c.965_966delCT	Exon 6	NP_003891.1:p.Pro322 fs	LIR	
NM_003900.4:c.1001_1003delGAG	Exon 7	NP_003891.1:p.Gly334del	LIR	
NM_003900.4:c.1045T>A	Exon 7	NP_003891.1:p.Ser349Thr	PEST2	
NM_003900.4:c.1084G>A	Exon 7	NP_003891.1:p.Glu362Lys		
NM_003900.4:c.1108T>C	Exon 7	NP_003891.1:p.Ser370Pro		rs143956614
NM_003900.4:c.1273G>A	Exon 8	NP_003891.1:p.Gly425Arg	UBA	
NM_003900.4:c.1277C>T	Exon 8	NP_003891.1:p.Ala426Val	UBA	

DNA quality or contamination were excluded, resulting in the final study population of 1,808 FTLD, 395 ALS, and 1,625 control individuals.

SQSTM1 sequencing

For the 1,808 FTLD, 395 ALS and 1,625 control individuals, the 8 coding exons and intron–exon boundaries were amplified by PCR of gDNA, followed by Sanger sequencing (NM_003900.4, primers available on request). Sequences were analyzed using the software package NovoSNP [39] and confirmed by visual inspection of the DNA sequence traces. Available WES data on an additional 2,274 German controls were checked for *SQSTM1* coding variants [10, 40].

Genetic variations were further verified in the Database of Single-Nucleotide Polymorphisms (dbSNP Build ID 137; URL http://www.ncbi.nlm.nih.gov/SNP/); the exome variant server (EVS) of the National Heart, Lung, and Blood Institute GO Exome Sequencing Project (Seattle, WA, USA; URL http://evs.gs.washington.edu/EVS/), and the 1,000 Genomes project (URL http://www.1000genomes.org/). The effects of rare coding variations in *SQSTM1* on protein structure and function were predicted using PMUT (http://mmb2.pcb.ub.es:8080/PMut/), SNPs&Go (http://snps.uib.es/snps-and-go//snps-and-go.html) and Provean/SIFT (http://sift.jcvi.org/www/SIFT_enst_submit.html).

Statistical analysis

We determined rare variants as genetic variants with an MAF <0.01 and performed a rare variant burden analysis following a stepwise approach. Alleles of all rare variants were first collapsed across the entire protein. Subsequently, calculations were repeated for rare variants associated with functional domains only. *SQSTM1* functional domains were determined according to [11] (Fig. 1b). Finally, rare variant data were calculated for each of the seven protein domains independently.

Calculations were performed in our source dataset and in a meta-analysis considering all published datasets generated by full exonic sequencing of *SQSTM1* in both FTLD patient and control groups [7, 17, 28] combined with the present study. Overall allele frequencies between patients and control individuals were compared using χ^2 statistics. In the per domain analyses p values were corrected for seven tests, corresponding to the seven functional domains. Rare variant association analysis was limited to the FTLD cohort due to the insufficient power of the ALS cohort to obtain significant association.

Neuropathology of *SQSTM1*

Formalin fixed, paraffin-embedded tissue blocks from neocortical areas, basal ganglia, thalamus, hippocampus,

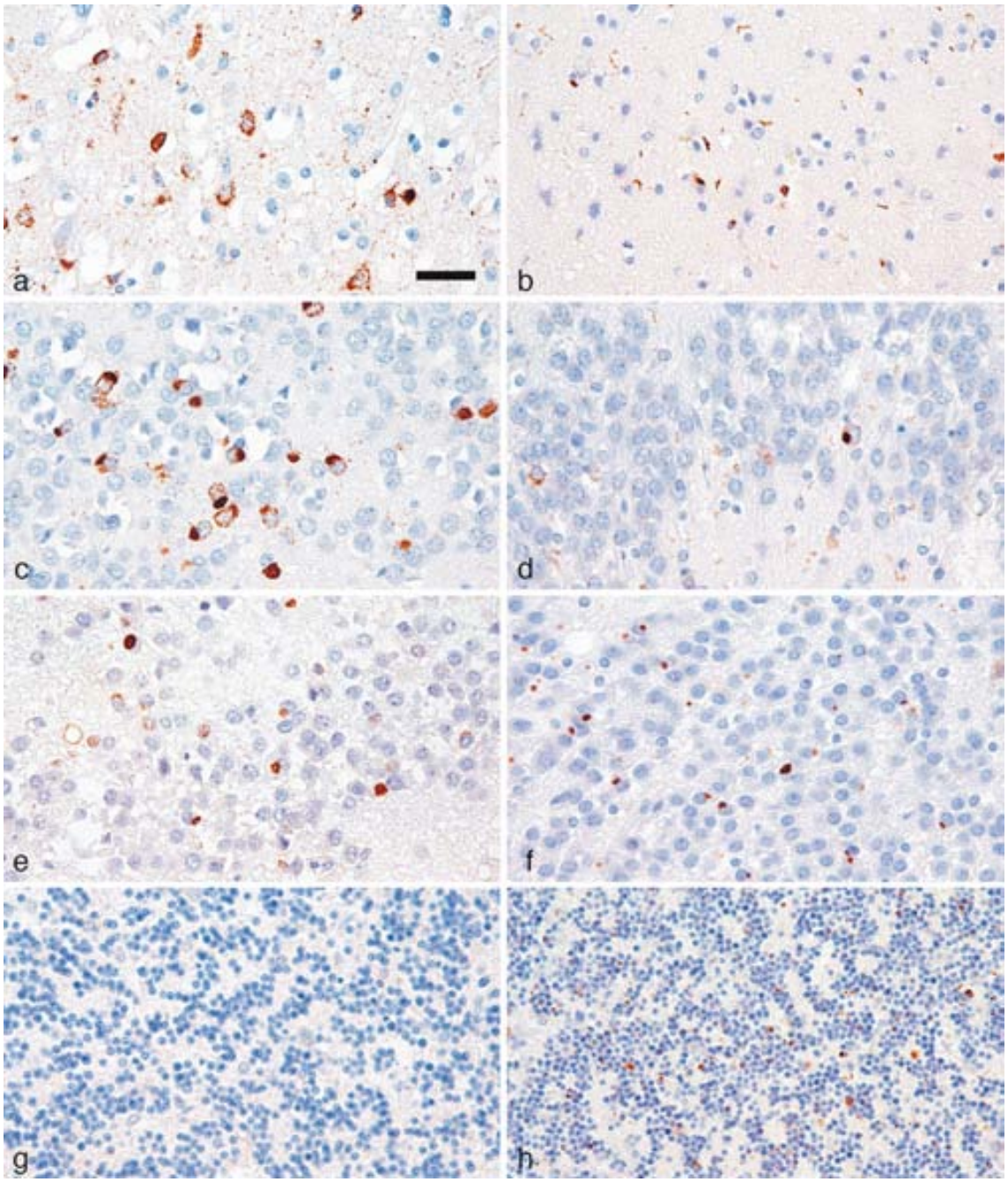


Fig. 2 Neuropathology observed in *SQSTM1* mutation carriers. Immunostaining for phospho-TDP-43 in the temporal cortex (**a**, **b**) and in the granule cells of the dentate gyrus (**c**, **d**) in a patient with a *SQSTM1* p.(Glu396*) mutation (**a**, **c**) and a second patient with a *SQSTM1* p.Arg212Cys—*C9orf72* double mutation (**b**, **d**). Notably, p62 immunoreactivity is less in the dentate gyrus (**e**, **f**) and lacking in the cerebellar granule cell layer (**g**, **h**) in the p.(Glu396*) case (**e**, **g**) as compared to the p.Arg212Cys patient with the additional *C9orf72* repeat expansion mutation (**f**, **h**). Scale bar represents 25 μm for all

brainstem and cerebellum were evaluated. In addition to Hematoxylin and Eosin staining, the following monoclonal (mouse) antibodies were used for immunohistochemistry: monoclonal anti-p62 (1:1,000, BD Transduction, Lexington KY, USA), anti-tau AT8 (pS202/pT205, 1:200, Pierce Biotechnology, Rockford, IL, USA), anti-phospho-TDP-43 (pS409/410, 1:2,000, Cosmo Bio, Tokyo, Japan), anti-α-synuclein (1:2,000, clone 5G4, Roboscreen, Leipzig, Germany; specific for disease-associated form), anti-Aβ (1:50, clone 6F/3D, Dako, Glostrup, Denmark). The DAKO EnVision© detection kit, peroxidase/DAB, rabbit/mouse (Dako, Glostrup,

Table 4 *SQSTM1* mutations published in previous studies

Mutation	Functional domain	FTLD	ALS	Origin	Study
p.Ala53Thr	PB1		1	Japanese	Hirano et al. [11]
p.Met87Val	PB1		1	French	Teyssou et al. [33]
p.Val90Met[a]	PB1		1	Japanese	Shimizu et al. [29]
p.Lys102Glu	PB1		1	French	Teyssou et al. [33]
p.Arg110Cys		2	1	French	Le Ber et al. [17]
p.Pro228Leu	TRAF6		1	Euro-American	Fecto et al. [7]
p.Lys238del	TRAF6		1	Euro-American	Fecto et al. [7]
p.Val259Leu		1		Italian	Rubino et al. [28]
p.Ser318Pro			1	Euro-American	Fecto et al. [7]
p.Arg321His	LIR	2	1	French	Le Ber et al. [17]
p.Lys344Glu	LIR	1		Italian	Rubino et al. [28]
p.Pro348Leu	PEST2		1	Italian	Rubino et al. [28]
p.Ala381Val		1	1	French	Le Ber et al. [17]
p.Pro387Leu	UBA	1		French	Le Ber et al. [17]
p.G351_P388del	UBA		1	French	Teyssou et al. [33]
p.Gly411Ser	UBA		1	Euro-American	Fecto et al. [7]

Mutations published in previous studies [7, 11, 17, 28, 29, 33] absent from published control persons or control persons tested in the present study are listed

[a] This mutation was compound heterozygous with p.Val153Ile in one Japanese ALS patient [29], p.Val153Ile was also observed in 3 control individuals of the present study. Fecto et al. [7] tested 546 ALS and 724 controls. Rubino et al. [28] tested 170 FTLD, 124 ALS, and 145 controls. Teyssou et al. [33] tested 164 ALS and 360 controls. Hirano et al. [11] tested 61 ALS and 500 controls. Le Ber et al. [17] tested 188 FTLD, 164 ALS, and 352 controls. Shimizu et al. [29] tested 1 ALS and 189 controls

Denmark) was used for visualization of the antibody reactions.

Results

Information on family history was available for 76.2 % (1,378/1,808) of the FTLD patients and 83.0 % (328/395) of ALS patients (Supplementary table 1). In the FTLD group, 35.2 % (636/1,808) had a positive family history while 41.2 % (744/1,808) were considered sporadic patients. In the ALS group, 14.9 % (59/395) had a family history of disease while 68.1 % (269/395) were sporadic patients. Onset age distribution was 63 ± 9.9 years in the FTLD group and 58 ± 13.7 years in the ALS group compared to an age at inclusion of 66 ± 12.4 years in the control group (Supplementary table 1).

Mutation screen of *SQSTM1* in FTLD

In total we identified 25 rare, heterozygous variations that affected the coding region of *SQSTM1,* including 23 missense mutations, 1 nonsense mutation and one small in-frame deletion (Tables 1, 2, Supplementary table 2; Fig. 1). Fifteen mutations were present in 16 patients and were absent from 3,899 control individuals (Table 1). The majority of the 15 mutations involved highly conserved amino acid residues in SQSTM1 and were predicted to be pathogenic (Fig. 1a, Supplementary table 3). Among the 16 mutation carriers, 7 had a positive family history of disease and 2 patients had concomitant ALS (Table 1). Nine patients carried a novel mutation not previously associated with FTLD, ALS or PDB (p.Ala16Val, p.Asp80Glu, p.Arg212Cys, p.Gly219Val, p.Ser226Pro, p.Pro232Thr, p.Glu280del, p.Asp329Gly and p.Thr430Pro) (Fig. 1b). The remaining six mutations had been reported in ALS (p.Val90Met, p.Pro228Leu and p.Pro348Leu) [7, 28, 29], FTLD (p.Arg321His, p.Pro387Leu) [17] or PDB (p.Pro387Leu, p.(Glu396*)) [28] (Fig. 1b). Except for p.Ala16Val, p.Arg212Cys and p.Gly219Val, all other mutations were located in a predicted functional protein domain of SQSTM1/p62 (Fig. 1b). In addition to the patient-only mutations, we identified another 10 missense mutations (p.Ala17Val, p.Ala33Val, p.Lys103Arg, p.Ala117Val, p.Pro118Ser, p.Lys238Glu, p.Glu274Asp, p.Arg321Cys, p.Pro392Leu, p.Pro439Leu) that were also present in control individuals at low frequency (Table 2). Except for p.Glu274Asp (MAF of 0.024), all were present in less than 1 % of control individuals. p.Ala17Val was a novel variation not previously reported in FTLD, ALS and/or PDB patients. We identified another 21 variants present in control individuals only (Table 3). When considering all rare

variants (MAF <0.01) observed in FTLD patients an overall frequency was calculated of 3.2 % (58/1,808).

Mutation screen of *SQSTM1* in ALS

For comparison with our FTLD findings, we analyzed the 395 ALS patients that were concurrently ascertained within the EU EOD consortium (Fig. 1). We identified three novel missense mutations present only in the ALS patients (p.Arg107Trp, p.Asp129Asn and p.Asp258Asn) (Table 1) and five other missense mutations (p.Val153Ile, p.Lys238Glu, p.Glu274Asp, p.Arg321Cys and p.Pro392Leu) also present in control individuals at low frequency (Table 2, Supplementary table 2).

Association of rare *SQSTM1* variants

We performed a burden analysis collapsing all rare variants with an MAF <0.01 across the whole protein. The same frequency of rare alleles was calculated for FTLD patients 0.016 (58/3,616 rare variant alleles) as for control individuals 0.016 (125/7,798; p value = 0.997, n.s.). We repeated the same analysis for rare variants present in functional domains only, resulting in a shift in allele frequencies of 0.014 (52/3,616) in FTLD versus 0.011 (84/7,798) controls, although not reaching statistical significance (p = 0.098). To increase genetic power, we considered all published datasets that were generated by full exonic sequencing of *SQSTM1* in both FTLD patient and control groups [7, 17, 28] and included the data in a meta-analysis with the present study comprising mutant allele frequencies in 4,332 FTLD and 10,240 control alleles. Burden analysis of all rare variants across the protein showed again no increase in patients (70/4,332 = 0.016 in FTLD versus 142/10,240 = 0.014 in controls; p value = 0.291, n.s.), but for the rare variants associated with functional domains a marked significant increase could be calculated of 0.014 (61/4,332 rare variant alleles) in FTLD patients versus 0.009 (97/10,240) in control individuals (relative risk overall (RR) = 1.49 [95 % CI 1.08–2.06]; p value = 0.014).

Subsequently, we investigated which of the functional domains were most contributing to the association. Rare variant data were calculated for each of the seven protein domains. In our source, FTLD cohort significant association was found with the C-terminal ubiquitin-associated (UBA) domain in patients (21/3,616 = 0.006 rare variant alleles) versus control individuals (20/7,798 = 0.003) (RR = 2.27 [95 % CI 1.23–4.20]; p value = 0.007, corrected p value = 0.049). In the meta-dataset, statistically significant clustering in the UBA domain was confirmed in FTLD (23/4,332 = 0.005 rare variant alleles) versus control individuals (25/10,240 = 0.002) (RR = 2.18 [95 % CI 1.24–3.85]; p value = 0.006, corrected p value = 0.042). In addition, also the LC3 interaction region (LIR) domain showed suggestive clustering of rare variants in FTLD (8/4,332 = 0.002) versus control individuals (5/10,240 = 0.0005) (RR = 3.79 [95 % CI 1.24–11.58]; p value = 0.012, corrected p value = 0.084).

Histopathology associated with *SQSTM1* mutations

Detailed histopathology was performed on autopsy brain of two carriers of a nonsense mutation p.(Glu396*) located in the UBA domain and a missense mutation p.Arg212Cys in a patient who also carried a *C9orf72* repeat expansion. Both cases showed widespread phospho-TDP-43 immunoreactive inclusions in neurons and glial cells. In the p.(Glu396*) carrier, frontal and temporal cortical areas mainly displayed neuronal cytoplasmic inclusions. Neuronal cytoplasmic inclusions were further abundant in the granule cells of the dentate gyrus. In addition, many glial phospho-TDP-43 inclusions were seen in the white matter. Although this brain suffered severe ischemic/hypoxic damage, inclusion bodies were clearly recognized as a distinctive feature. In contrast, neuropathological features of the p.Arg212Cys carrier revealed alterations reminiscent of the *C9orf72* pathology, including the characteristic p62-positive but phospho-TDP-43-negative inclusions in the hippocampal pyramidal layer and cerebellar granule cells (Fig. 2).

Discussion

Previous studies in ALS (total n = 895) [7, 11, 28, 33], and FTLD (total n = 358) [17, 28] estimated the frequency of *SQSTM1* coding mutations at 2.42–3.28 % in ALS and 1.76–2.13 % in FTLD. Because of this relatively low prevalence, large patient and control groups are needed to obtain a reliable estimation of the mutation frequency of *SQSTM1*. Mutations in *SQSTM1* were first described in patients with PDB [16]. Up to one-third of patients with familial PDB are explained by a *SQSTM1* mutation, with p.Pro392Leu as the most commonly observed mutation [4]. A relationship between PDB and FTLD has been previously demonstrated by the identification of causal mutations in *VCP* in families segregating the rare syndrome of inclusion body myopathy with Paget disease of bone and frontotemporal lobar degeneration (IBMPFD) [38]. Later studies demonstrated that *VCP* mutations express significant clinical heterogeneity and patients can present with all, a combination of two or just one of the three core phenotypes of IBMPFD [24, 36]. Also more recently, WES identified a *VCP* mutation segregating in a family with ALS and subsequent screenings in ALS patients identified additional *VCP* mutations [12].

In this study, we analyzed a European cohort of 1,808 FTLD patients for mutations in *SQSTM1* and identified 25 mutations in the coding sequence of which 15 mutations in 16 patients were absent from 3,899 control persons. This resulted in a mutation frequency for *SQSTM1* of 0.9 % overall and 1.1 % in familial FTLD patients. Some of the mutations are listed in dbSNP Short Genetic Variations database, the 1,000 Genomes Project database or the exome variant server (EVS), yet at very low frequencies of MAF ≤0.001 (Supplementary table 2). The *SQSTM1* mutation frequency that we calculated in FTLD is lower than in previous studies. This can potentially be explained by the fact that we excluded some of the previously reported patient-only mutations from our calculations since they were present in our extended control group, though at low frequencies <0.01 % (Fig. 1b). This was the case for p.Arg321Cys, p.Ser370Pro, p.Gly425Arg [7]; p.Ala33Val, p.Pro392Leu [7, 17]; p.Lys238Glu, p.Glu319Lys [28]; p.Pro439Leu [11]; and p.Val153Ile [7, 29]. In Table 4, all *SQSTM1* mutations are listed that have been published [7, 11, 17, 28, 29, 33] and were absent from published and tested control persons. When we considered all rare variants (MAF < 0.01), indifferent of whether or not they appeared in control individuals, we obtained a mutation frequency of 3.2 % in FTLD patients which is the same as calculated in the pooled data analysis of the present and published FTLD cohorts [17, 28]. Overall, no statistical association in patients versus control individuals was observed when pooling all rare *SQSTM1* variants, not in our study nor in the meta-analysis with all published patient and control datasets [7, 17, 28]. Yet, when considering only domain-associated variants, a trend toward association was observed in our study and a significant increase in patients was reached in the more powerful meta-analysis dataset. Per domain analysis indicated that association was driven by the UBA domain and possibly also the LIR domain. The C-terminal UBA domain, which is primarily affected in PDB, contained significant more variants in FTLD patients when compared to control individuals in both our study and the meta-analysis (0.5 versus 0.2 %) ($RR_{meta} = 2.18$ [95 % CI 1.24–3.85]; nominal p $value_{meta} = 0.006$; corrected p $value_{meta} = 0.042$). In the LIR domain through which SQSTM1 binds the autophagy effector protein LC3, 0.2 % of FTLD patients carried a mutation versus 0.05 % of control individuals ($RR_{meta} = 3.79$ [95 % CI 1.24–11.58]; nominal p $value_{meta} = 0.012$; corrected p $value_{meta} = 0.084$). In contrast to Rubino and colleagues who sequenced up to 1,700 bp into the *SQSTM1* promoter and detected 4 variants in 4 out of 170 FTLD patients absent from 145 control individuals (c. −1,221 G>A, c. −1,165 C>T, c. −1,153 C>G, c. −673 T>C) [28], we did not investigate the *SQSTM1* promoter for rare variants.

The pathogenicity of *SQSTM1* mutations, even those that were absent from control individuals, remains unclear at this stage. Most of the patient-only mutations involved highly conserved amino acid residues, with 75 % of the mutations residing in predicted functional domains. In silico prediction programs of amino acid changes indicated that all mutations might be pathogenic by one or more of the in silico programs. Also, the three mutations that resided outside predicted domains were scored as deleterious by one to all four in silico programs. Of course, these in silico predictions are merely indicative and should be interpreted with caution. In contrast to PDB-associated mutations which cluster at the UBA domain [11, 16], FTLD mutations are mostly distributed throughout the protein as also previously reported for ALS, though significantly more mutations seem to be located in the UBA domain. Further, Le Ber and colleagues provided evidence for co-segregation with FTLD for two mutations in the UBA domain, p.Pro387Leu and p.Pro392Leu [17]. The p.Pro387Leu mutation was also present in two unrelated Italian FTLD patients in our study who did not share a common haplotype, suggesting a recurrent de novo mutation. No DNA of relatives of these two mutation carriers was available to test for co-segregation. Three mutation carriers (p.Ala16Val, p.Arg212Cys, p.Gly219Val) also carried a pathogenic *C9orf72* repeat expansion. At this stage, we have no clear indication that the co-existence of these mutations influences clinical expression of disease. Onset ages were not different from those in patients carrying only a *SQSTM1* mutation. In a Japanese sporadic late-onset ALS patient with pathology confirmed predominant lower motor neuron disease, a compound heterozygous *SQSTM1* mutation was detected, p.[Val90Met(;)Val153Ile] [29]. We did not observe compound heterozygous *SQSTM1* mutation carriers, though both mutations present in the Japanese patient were also observed in our study, p.Val90Met in one FTLD patient and p.Val153Ile in one other FTLD patient and three control individuals. One could hypothesize that the rare *SQSTM1* variants act as high to intermediate penetrant risk alleles and in case of the Japanese patient both had to be present to exert the clinical profile of ALS.

The hallmark lesions of FTLD-TDP are neuronal and glial inclusions with positive immunoreactivity for phospho-TDP-43. In addition to sporadic patients, until now mutations in *VCP*, *GRN* and *C9orf72* have been associated consistently with TDP-43 pathology, while TARDBP mutations are less frequently observed in FTLD [19]. Here, we provide histopathological evidence that *SQSTM1* mutations are associated with TDP-43 pathological inclusions. Interestingly, the patient with only a *SQSTM1* mutation (p.Glu396*) showed widespread neuronal and glial phospho-TDP-43 pathology. The patient (p.Arg212Cys) who carried also a *C9orf72* mutation showed *C9orf72*-related pathology without other noticeable distinctive features. Potential explanations could

be that the *C9orf72* mutation masked the effect of the *SQSTM1*mutation, or that the *C9orf72* pathology dominates the *SQSTM1* pathology. Also the *SQSTM1* p.Arg212Cys mutation may well have a lower penetrance and be associated with more subtle pathological changes than the (p.Glu396*) mutation located in the UBA domain. These questions merit further investigation and more *SQSTM1*-positive pathologies will need to be evaluated to resolve these issues. Since mutations in *SQSTM1* were first linked to PDB, we carefully reexamined medical records of all carriers of patient-only *SQSTM1* mutations for indications of (sub) clinical symptoms of PDB. Only in one Portuguese patient carrying the p.Pro232Thr mutation located in the TRAF6 domain, the diagnosis of possible PDB could be made at hindsight. We, however, cannot exclude that the presence of PDB might have been missed in the other patients since PDB often remains asymptomatic and a diagnosis requires radiography. Likewise we cannot exclude that some of the control individuals, who were selected based on absence of dementia-related symptoms, may have been at risk for/or suffered from PDB, in particular, those with the PDB-associated *SQSTM1* p.P392L mutation.

In conclusion, our study represents one of the largest screening efforts of FTLD patients for mutations in *SQSTM1*. Further, we combined the data from our screening with that of published datasets in a meta-analysis of rare *SQSTM1* variants in a total of 4,432 FTLD and 10,240 control alleles. Both our study and the meta-analysis calculated a mutation frequency of 3.2 % in FTLD patients. Also, the meta-analysis suggested that rare mutations clustering in the UBA domain of *SQSTM1* may influence disease susceptibility by doubling the risk for FTLD. Histopathology of autopsied brain of *SQSTM1* mutation carriers demonstrated a widespread neuronal and glial phospho-TDP-43 pathology. Taken together, our findings provide additional evidence that *SQSTM1* is implicated in the pathogenicity of FTLD/ALS spectrum diseases.

Acknowledgments The authors are grateful to the personnel of the Genetic Service Facility for their support of the genetic analyses and to the different neurological centers for their contribution to the diagnosis and sampling of patients. The data generation for this paper was in part funded by the MetLife Award for Medical Research to C.V.B., USA; the Belgian Science Policy Office (BELSPO) Interuniversity Attraction Poles program, the Flemish government support to the European Initiative on Centers of Excellence in Neurodegeneration (CoEN), the Flemish Government initiated Methusalem excellence program, the Alzheimer Research Foundation (SAO/FRA), the Medical Foundation Queen Elisabeth, the Research Foundation Flanders (FWO), the Agency for Innovation by Science and Technology Flanders (IWT) and the University of Antwerp Research Fund, Belgium. The FWO provided a postdoctoral fellowship to J.v.d.Z. and a clinical investigatorship to P.V.D. *The Brescia IRCCS Fatebenefratelli site* was funded by the Ricerca Corrente, Italian Ministry of Health. R.G. was funded by Fondazione CARIPLO, Grant Number 2009-2633. *The Barcelona ACE site* thank the patients and controls who participated in this project. We are indebted to Trinitat Port-Carbó and her family who are supporting Fundació ACE research programs. *The Lisbon site* acknowledges a grant by Grunenthal. *The Florence site* acknowledges Prin 2010-prot. 2010PWNJXK; Cassa di Rispario di Firenze e Cassa di Risparmio di Pistoia e Pescia. *The Stockholm site* was financially supported by the Programme in Neuroscience at Karolinska Institutet (StratNeuro); the regional agreement on medical training and clinical research (ALF) between Stockholm County Council and Karolinska Institutet; Swedish Alzheimer Foundation; Swedish Research Council; Karolinska Institutet PhD-student funding; King Gustaf V and Queen Victoria's Free Mason Foundation; the Gun and Bertil Stohne's Foundation, Foundation for Old Servants; Clinicians including Dr Vesna Jelic and Anne Börjesson-Hanson. *For the Munich Institute of Human Genetics site* H.P. was supported by the E-Rare project GENOMIT (01GM1207), and the German Network for mitochondrial disorders (mitoNET 01GM1113C). T.B.H. was supported by the NBIA disorders association. *The Barcelona Sant Pau site* was in part funded by a grant from the Spanish Ministry of Economy and Competitiveness (Grant Number PI12/01311) and CIBERNED. *The Pamplona Center for Applied Medical Research* is indebted the UTE project from the Foundation for Applied Medical Research (FIMA) and CIBERNED. *The Barcelona Neurological Tissue Bank site* is indebted to the Neurological Tissue Bank of the IDIBAPS Biobanc for sample and data procurement. The Neurological Tissue Bank acknowledges all brain donors and relatives for generous brain donation for research and referring physicians. *The Czech site* was partially supported by grant IGA NT 12094-5 of Grant Agency of the Czech Ministry of Health. *The Verona site* was in part supported by Fondazione Cariverona (Grant Number 2009.1026 "Cognitive and behavioural disability in dementia and psychosis" to GMF). *The Liege site* was funded by the FNRS.

Conflict of interest The authors report no disclosure with regard to the reported findings.

Appendix

BELNEU consortium Jonathan Baets (Department of Molecular Genetics, VIB, Antwerp, Belgium; Institute Born-Bunge, University of Antwerp, Antwerp, Belgium; Antwerp University Hospital, Edegem, Belgium); Dirk Nuytten (Hospital Network Antwerp Stuivenberg, Antwerp, Belgium); Jan De Bleecker (University Hospital Ghent, Ghent, Belgium); Jan Versijpt, Alex Michotte (University Hospital Brussels, Brussels, Belgium); Adrian Ivanoiu (Saint-Luc University Hospital, Brussels, Belgium); Olivier Deryck, Bruno Bergmans (AZ Sint-Jan Brugge, Bruges, Belgium); Christiana Willems (Jessa Hospital, Hasselt, Belgium).

EU EOD consortium Anna Paterlini (Proteomics Unit, IRCCS Istituto Centro San Giovanni di Dio Fatebenefratelli, Brescia, Italy); Elena Alonso (Neurogenetics Laboratory, Division of Neurosciences, Center for Applied Medical Research, Universidad de Navarra, Pamplona, Spain);

Manuel Seijo-Martínez (Department of Neurology, Hospital do Salnés, Pontevedra, Spain); Ramon Rene, Jordi Gascon, Jaume Campdelacreu (Department of Neurology, Hospital de Bellvitge, Barcelona, Spain); Madalena Martins, Mafalda Matos, André Janeiro, Ana Verdelho (Faculty of Medicine and Institute of Molecular Medicine, University of Lisbon, Portugal); Irene Piaceri (Department of Neurosciences, Psychology, Drug Research and Child Health (NEUROFARBA) University of Florence, Florence, Italy); Jenny Björkström, Anne Kinhult Ståhlbom, Marie Fallström (Department of Geriatric Medicine, Genetics unit, Karolinska University Hospital, Stockholm, Sweden); Charlotte Forsell, Anna-Karin Lindström, Lena Lilius (Karolinska Institutet, Department of Neurobiology, Care Sciences and Society (NVS), KI-Alzheimer Disease Research Center, Stockholm, Sweden); Inger Nennesmo (Department of Clinical Pathology and Cytology, Karolinska University Hospital, Stockholm, Sweden); Laura Fratiglioni (Aging Research Center, Department of Neurobiology, Care Sciences and Society (NVS), Karolinska Institutet and Stockholm University, Stockholm, Sweden);Tamara Eisele (Department of Psychiatry and Psychotherapy, Technische Universität München, 81675 München, Germany); Thomas Wieland (Institute of Human Genetics, Technische Universität München, 81675 Munich, Germany); Ricard Rojas-García, Marc Suárez-Calvet (Department of Neurology, IIB Sant Pau, Hospital de la Santa Creu i Sant Pau, Universitat Autònoma de Barcelona, Barcelona, Spain; Center for Networker Biomedical Research in Neurodegenerative Diseases (CIBERNED), Madrid, Spain); João Massano (Department of Neurology Centro Hospitalar São João, Portugal; Department of Clinical Neuroscience and Mental Health, Faculty of Medicine University of Porto, Porto, Portugal); Maria Helena Ribeiro (Faculty of Medicine, University of Coimbra, Coimbra, Portugal); Catarina Oliveira (Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal); Jose L Molinuevo, Anna Antonell (Alzheimer's disease and other cogntive disorders unit. Neurology department, Hospital); Robert Rusina, Zdenek Rohan (Department of Pathology and Molecular Medicine, Thomayer Hospital, Prague, Czech Republic); Robert Rusina (Center of Clinical Neurosciences, Department of Neurology, First Medical Faculty, Charles University in Prague, Czech Republic); Tiziana Cavallaro (Department of Neuroscience, AOUI Verona, Verona, Italy).

References

1. Al-Sarraj S, King A, Troakes C et al (2011) p62 positive, TDP-43 negative, neuronal cytoplasmic and intranuclear inclusions in the cerebellum and hippocampus define the pathology of C9orf72-linked FTLD and MND/ALS. Acta Neuropathol 122:691–702. doi:10.1007/s00401-011-0911-2
2. Arai T, Nonaka T, Hasegawa M et al (2003) Neuronal and glial inclusions in frontotemporal dementia with or without motor neuron disease are immunopositive for p62. Neurosci Lett 342:41–44. doi:10.1016/S0304-3940(03)00216-7
3. Brooks BR, Miller RG, Swash M, Munsat TL (2000) El Escorial revisited: revised criteria for the diagnosis of amyotrophic lateral sclerosis. Amyotroph Lateral Scler Other Motor Neuron Disord 32:505–514
4. Chung PYJ, Beyens G, Guañabens N et al (2008) Founder effect in different European countries for the recurrent P392L SQSTM1 mutation in Paget's disease of bone. Calcif Tissue Int 83:34–42. doi:10.1007/s00223-008-9137-2
5. Cruts M, Gijselinck I, Van Langenhove T et al (2013) Current insights into the C9orf72 repeat expansion diseases of the FTLD/ALS spectrum. Trends Neurosci 36:450–459. doi:10.1016/j.tins.2013.04.010
6. Deng HX, Zhai H, Bigio EH et al (2010) FUS-immunoreactive inclusions are a common feature in sporadic and non-SOD1 familial amyotrophic lateral sclerosis. Ann Neurol 67:739–748
7. Fecto F, Yan J, Vemula SP et al (2011) SQSTM1 mutations in familial and sporadic amyotrophic lateral sclerosis. Arch Neurol 68:1440–1446. doi:10.1001/archneurol.2011.250
8. Gelpi E, van der Zee J, Turon Estrada A et al (2014) TARDBP mutation p.Ile383Val associated with semantic dementia and complex proteinopathy. Neuropathol Appl Neurobiol 40:225–230. doi:10.1111/nan.12063
9. Gennari L, Gianfrancesco F, Di Stefano M et al (2010) SQSTM1 gene analysis and gene-environment interaction in Paget's disease of bone. J Bone Miner Res 25:1375–1384. doi:10.1002/jbmr.31
10. Haack TB, Kopajtich R, Freisinger P et al (2013) ELAC2 mutations cause a mitochondrial RNA processing defect associated with hypertrophic cardiomyopathy. Am J Hum Genet 93:211–223. doi:10.1016/j.ajhg.2013.06.006
11. Hirano M, Nakamura Y, Saigoh K et al (2013) Mutations in the gene encoding p62 in Japanese patients with amyotrophic lateral sclerosis. Neurology 80:458–463. doi:10.1212/WNL.0b013e31827f0fe5
12. Johnson JO, Mandrioli J, Benatar M et al (2010) Exome sequencing reveals VCP mutations as a cause of familial ALS. Neuron 68:857–864
13. Kimonis VE, Fulchiero E, Vesa J, Watts G (2008) VCP disease associated with myopathy, Paget disease of bone and frontotemporal dementia: review of a unique disorder. Biochim Biophys Acta 1782:744–748. doi:10.1016/j.bbadis.2008.09.003
14. Kurihara N, Hiruma Y (2007) Mutation of the sequestosome 1 (p62) gene increases osteoclastogenesis but does not induce Paget disease. J Clin Invest 117:133–142. doi:10.1172/JCI28267
15. Kuusisto E, Salminen A, Alafuzoff I (2001) Ubiquitin-binding protein p62 is present in neuronal and glial inclusions in human tauopathies and synucleinopathies. Neuroreport 12:2085–2090
16. Laurin N, Brown JP, Morissette J, Raymond V (2002) Recurrent mutation of the gene encoding sequestosome 1 (SQSTM1/p62) in Paget disease of bone. Am J Hum Genet 70:1582–1588. doi:10.1086/340731
17. Le Ber I, Camuzat A, Guerreiro R, Campion D (2013) SQSTM1 mutations in French patients with frontotemporal dementia or frontotemporal dementia with amyotrophic lateral sclerosis. JAMA Neurol 70:1403–1410. doi:10.1001/jamaneurol.2013.3849
18. Litvan I, Agid Y, Calne D et al (1996) Clinical research criteria for the diagnosis of progressive supranuclear palsy (Steele-Richardson-Olszewski syndrome): report of the NINDS-SPSP international workshop. Neurology 47:1–9
19. Mackenzie IRA, Neumann M, Baborie A et al (2011) A harmonized classification system for FTLD-TDP pathology. Acta Neuropathol 122:111–113. doi:10.1007/s00401-011-0845-8
20. Mizuno Y, Amari M, Takatama M et al (2006) Immunoreactivities of p62, an ubiqutin-binding protein, in the spinal anterior horn

cells of patients with amyotrophic lateral sclerosis. J Neurol Sci 249:13–18. doi:10.1016/j.jns.2006.05.060
21. Mori K, Arzberger T, Grässer FA et al (2013) Bidirectional transcripts of the expanded C9orf72 hexanucleotide repeat are translated into aggregating dipeptide repeat proteins. Acta Neuropathol 126:881–893. doi:10.1007/s00401-013-1189-3
22. Mori K, Weng S-M, Arzberger T et al (2013) The C9orf72 GGGGCC repeat is translated into aggregating dipeptide-repeat proteins in FTLD/ALS. Science 339:1335–1338. doi:10.1126/science.1232927
23. Nakaso K, Yoshimoto Y, Nakano T et al (2004) Transcriptional activation of p62/A170/ZIP during the formation of the aggregates: possible mechanisms and the role in Lewy body formation in Parkinson's disease. Brain Res 1012:42–51. doi:10.1016/j.brainres.2004.03.029
24. Nalbandian A, Donkervoort S, Dec E et al (2011) The multiple faces of valosin-containing protein-associated diseases: inclusion body myopathy with Paget's disease of bone, frontotemporal dementia, and amyotrophic lateral sclerosis. J Mol Neurosci 45:522–531. doi:10.1007/s12031-011-9627-y
25. Neary D, Snowden JS, Gustafson L et al (1998) Frontotemporal lobar degeneration: a consensus on clinical diagnostic criteria. Neurology 51:1546–1554
26. Pankiv S, Clausen TH, Lamark T et al (2007) p62/SQSTM1 binds directly to Atg8/LC3 to facilitate degradation of ubiquitinated protein aggregates by autophagy. J Biol Chem 282:24131–24145. doi:10.1074/jbc.M702824200
27. Rascovsky K, Hodges JR, Knopman D et al (2011) Sensitivity of revised diagnostic criteria for the behavioural variant of frontotemporal dementia. Brain 134:2456–2477. doi:10.1093/brain/awr179
28. Rubino E, Rainero I, Chiò A et al (2012) SQSTM1 mutations in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Neurology 79:1556–1562. doi:10.1212/WNL.0b013e31826e25df
29. Shimizu H, Toyoshima Y, Shiga A et al (2013) Sporadic ALS with compound heterozygous mutations in the SQSTM1 gene. Acta Neuropathol 126:453–459. doi:10.1007/s00401-013-1150-5
30. Synofzik M, Born C, Rominger A, et al. (2013) Targeted high-throughput sequencing identifies a TARDBP mutation as a cause of early-onset FTD without motor neuron disease. Neurobiol Aging 35:1212.e1–5. doi:10.1016/j.neurobiolaging.2013.10.092
31. Synofzik M, Maetzler W, Grehl T et al (2012) Screening in ALS and FTD patients reveals 3 novel UBQLN2 mutations outside the PXX domain and a pure FTD phenotype. Neurobiol Aging 33(2949):e13–e17. doi:10.1016/j.neurobiolaging.2012.07.002
32. Tanji K, Zhang H-X, Mori F et al (2012) p62/sequestosome 1 binds to TDP-43 in brains with frontotemporal lobar degeneration with TDP-43 inclusions. J Neurosci Res 90:2034–2042. doi:10.1002/jnr.23081
33. Teyssou E, Takeda T, Lebon V et al (2013) Mutations in SQSTM1 encoding p62 in amyotrophic lateral sclerosis: genetics and neuropathology. Acta Neuropathol 125:511–522. doi:10.1007/s00401-013-1090-0
34. Troakes C, Maekawa S, Wijesekera L et al (2011) An MND/ALS phenotype associated with C9orf72 repeat expansion: abundant p62-positive, TDP-43-negative inclusions in cerebral cortex, hippocampus and cerebellum but without associated cognitive decline. Neuropathol Off J Japanese Soc Neuropathol 32:505–514. doi:10.1111/j.1440-1789.2011.01286.x
35. van der Zee J, Gijselinck I, Dillen L et al (2013) A Pan-European study of the C9orf72 repeat associated with FTLD: geographic prevalence, genomic instability, and intermediate repeats. Hum Mutat 34:363–373. doi:10.1002/humu.22244
36. van der Zee J, Pirici D, Van Langenhove T et al (2009) Clinical heterogeneity in 3 unrelated families linked to VCP p.Arg159His. Neurology 73:626–632
37. Van Langenhove T, van der Zee J, Van Broeckhoven C (2012) The molecular basis of the frontotemporal lobar degeneration-amyotrophic lateral sclerosis spectrum. Ann Med 44:817–828. doi:10.3109/07853890.2012.665471
38. Watts GDJ, Wymer J, Kovach MJ et al (2004) Inclusion body myopathy associated with Paget disease of bone and frontotemporal dementia is caused by mutant valosin-containing protein. Nat Genet 36:377–381
39. Weckx S, Del-Favero J, Rademakers R et al (2005) novoSNP, a novel computational tool for sequence variation discovery. Genome Res 15:436–442. doi:10.1101/gr.2754005
40. Zimprich A, Benet-Pagès A, Struhal W et al (2011) A mutation in VPS35, encoding a subunit of the retromer complex, causes late-onset Parkinson disease. Am J Hum Genet 89:168–175

Neurobiology of Aging xxx (2014) 1–7

Contents lists available at ScienceDirect

Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging

Investigation of the role of rare *TREM2* variants in frontotemporal dementia subtypes

Mathias Thelen [a], Cristina Razquin [b,c], Isabel Hernández [d], Ana Gorostidi [c,e], Raquel Sánchez-Valle [f], Sara Ortega-Cubero [b,c], Steffen Wolfsgruber [a,g], Dmitriy Drichel [g], Klaus Fliessbach [a,g], Tanja Duenkel [h], Marinella Damian [h], Stefanie Heilmann [i], Anja Slotosch [a], Martina Lennarz [a], Manuel Seijo-Martínez [j], Ramón Rene [k], Johannes Kornhuber [l], Oliver Peters [m], Christian Luckhaus [n], Holger Jahn [o], Michael Hüll [p], Eckart Rüther [q], Jens Wiltfang [q], Elena Lorenzo [b,c], Jordi Gascon [k], Alberto Lleó [c,r], Albert Lladó [f], Jaume Campdelacreu [k], Fermin Moreno [c,e,s], Hojjat Ahmadzadehfar [t], Dementia Genetics Spanish Consortium (DEGESCO), Juan Fortea [c,r], Begoña Indakoetxea [c,e,s], Michael T. Heneka [g,u], Axel Wetter [v], Maria A. Pastor [w,z], Mario Riverol [w], Tim Becker [g,x], Lutz Frölich [h], Lluís Tárraga [d], Mercè Boada [d], Michael Wagner [a,g], Frank Jessen [a,g], Wolfgang Maier [a,g], Jordi Clarimón [c,r], Adolfo López de Munain [c,e,s,y], Agustín Ruiz [d], Pau Pastor [b,c,w], Alfredo Ramirez [a,i,*]

[a] *Department of Psychiatry and Psychotherapy, University of Bonn, Bonn, Germany*
[b] *Neurogenetics Laboratory, Division of Neurosciences, Center for Applied Medical Research, University of Navarra School of Medicine, Pamplona, Spain*
[c] *Center for Network Biomedical Research into Neurodegenerative Diseases (CIBERNED), Instituto de Salud Carlos III, Madrid, Spain*
[d] *Memory Clinic of Fundaciò ACE, Institut Català de Neurociències Aplicades, Barcelona, Spain*
[e] *Neuroscience Area, BioDonostia Health Research Institute, San Sebastian, Spain*
[f] *Alzheimer's Disease and Other Cognitive Disorders Unit, Department of Neurology, Hospital Clinic, IDIBAPS, Barcelona, Spain*
[g] *German Center for Neurodegenerative Diseases (DZNE), Bonn, Germany*
[h] *Department of Geriatric Psychiatry, Central Institute of Mental Health, Medical Faculty Mannheim, University of Heidelberg, Heidelberg, Germany*
[i] *Institute of Human Genetics, University of Bonn, Bonn, Germany*
[j] *Department of Neurology, Hospital do Salnés, Pontevedra, Spain*
[k] *Department of Neurology, Hospital Universitari de Bellvitge, Barcelona, Spain*
[l] *Department of Psychiatry and Psychotherapy, Friedrich-Alexander-University of Erlangen-Nuremberg, Erlangen, Germany*
[m] *Department of Psychiatry, Charité, Berlin, Germany*
[n] *Department of Psychiatry and Psychotherapy, Medical Faculty, Heinrich-Heine University, Düsseldorf, Germany*
[o] *Department of Psychiatry, University of Hamburg, Hamburg, Germany*
[p] *Centre for Geriatric Medicine and Section of Gerontopsychiatry and Neuropsychology, Medical School, University of Freiburg, Freiburg, Germany*
[q] *Department of Psychiatry and Psychotherapy, University of Göttingen, Göttingen, Germany*
[r] *Department of Neurology, Hospital de la Santa Creu i Sant Pau, IIB-Sant Pau, Universitat Autònoma de Barcelona, Barcelona, Spain*
[s] *Department of Neurology, Donostia University Hospital, San Sebastian, Spain*
[t] *Department of Nuclear Medicine, University of Bonn, Bonn, Germany*
[u] *Clinical Neuroscience Unit, Department of Neurology, University of Bonn, Bonn, Germany*
[v] *Department of Radiology, University of Essen, Essen, Germany*
[w] *Department of Neurology, Clínica Universidad de Navarra, University of Navarra School of Medicine, Pamplona, Spain*
[x] *Institute for Medical Biometry, Informatics, and Epidemiology, University of Bonn, Bonn, Germany*
[y] *Department of Neurosciences, University of Basque Country, San Sebastian, Spain*
[z] *Neuroimaging Laboratory, Division of Neuroscience, Center for Applied Medical Research (CIMA), University of Navarra, Pamplona, Spain*

MT, CR, PP, and AR contributed equally to the study.

* Corresponding author at: Department of Psychiatry and Psychotherapy, University of Bonn, Sigmund-Freud-Strasse 25, Bonn D-53105, Germany. Tel.: +49 (0)228 287 19323; fax: +49 (0)228 287 16097.
E-mail address: alfredo.ramirez@ukb.uni-bonn.de (A. Ramirez).

0197-4580/$ – see front matter
http://dx.doi.org/10.1016/j.neurobiolaging.2014.06.018

ARTICLE INFO

Article history:
Received 28 April 2014
Received in revised form 13 June 2014
Accepted 16 June 2014

Keywords:
Alzheimer's disease
Frontotemporal dementia
Case-control studies
Genetic association studies

ABSTRACT

Frontotemporal dementia (FTD) is a clinically and genetically heterogeneous disorder. Rare *TREM2* variants have been recently identified in families affected by FTD-like phenotype. However, genetic studies of the role of rare *TREM2* variants in FTD have generated conflicting results possibly because of difficulties on diagnostic accuracy. The aim of the present study was to investigate associations between rare *TREM2* variants and specific FTD subtypes (FTD-S). The entire coding sequence of *TREM2* was sequenced in FTD-S patients of Spanish ($n = 539$) and German ($n = 63$) origin. Genetic association was calculated using Fisher exact test. The minor allele frequency for controls was derived from in-house genotyping data and publicly available databases. Seven previously reported rare coding variants (p.A28V, p.W44X, p.R47H, p.R62H, p.T66M, p.T96K, and p.L211P) and 1 novel missense variant (p.A105T) were identified. The p.R47H variant was found in 4 patients with FTD-S. Two of these patients showed cerebrospinal fluid pattern of amyloid beta, tau, and phosphorylated-tau suggesting underlying Alzheimer's disease (AD) pathology. No association was found between p.R47H and FTD-S. A genetic association was found between p.T96K and FTD-S ($p = 0.013$, odds ratio = 4.23, 95% Confidence Interval [1.17−14.77]). All 6 p.T96K patients also carried the *TREM2* variant p.L211P, suggesting linkage disequilibrium. The remaining *TREM2* variants were found in 1 patient, respectively, and were absent in controls. The present findings provide evidence that p.T96K is associated with FTD-S and that p.L211P may contribute to its pathogenic effect. The data also suggest that p.R47H is associated with an FTD phenotype that is characterized by the presence of underlying AD pathology.

1. Introduction

Frontotemporal dementia (FTD) is a heterogeneous disorder, which presents as 2 clinical subtypes: behavioral variant frontotemporal dementia (bv-FTD) and primary progressive aphasia (PPA) (McKhann et al., 2001). PPA is characterized by language impairment, and a recent consensus has established its subclassification into nonfluent variant (nfv-PPA), semantic variant (sv-PPA), and logopenic variant (lv-PPA) forms (Gorno-Tempini et al., 2011). The underlying neurodegenerative pathology of these FTD syndromes is heterogeneous and includes the presence of neuronal loss, hyperphosphorylated tau, deposit of TAR DNA-binding protein 43 (TDP43), and fused-in sarcome−positive inclusions in the frontal and temporal lobes (Cairns et al., 2007). Clinicopathologic correlation studies have demonstrated that patients with different FTD subtypes (FTD-S) may also be associated with the typical neuropathologic features of Alzheimer's disease (AD), in particular, patients with lv-PPA (Chare et al., 2014).

Research into familial and sporadic FTD has identified several causal genetic variants. These include (1) the hexanucleotide repeat expansion of the chromosome 9 open reading frame 72 gene (*C9ORF72*, OMIM 614260) (DeJesus-Hernandez et al., 2011; Dobson-Stone et al., 2013; Renton et al., 2011); (2) the granulin precursor gene (*GRN*, OMIM 138945) (Cruts et al., 2006); (3) the microtubule-associated protein tau gene (*MAPT*, OMIM 157140) (Hutton et al., 1998; Pastor et al., 2001); and (4) the triggering receptor expressed on myeloid cells 2 gene (*TREM2*, OMIM 605086) (Guerreiro et al., 2013b). In the case of *TREM2*, homozygous loss-of-function mutations were found in families with a rare recessive disease called polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy (also known as Nasu-Hakola disease) (Paloneva et al., 2002). This rare condition is characterized by early-onset progressive dementia and bone cysts. Interestingly, *TREM2* homozygous mutations were also identified in families affected by FTD-like dementia without bone cysts suggesting also an involvement of *TREM2* in FTD patients (Guerreiro et al., 2013b). However, other investigations of *TREM2* in FTD patients have generated conflicting results (Borroni et al., 2014; Cuyvers et al., 2014; Lattante et al., 2013; Ruiz et al., 2014). Despite these inconclusive data, rare variants in *TREM2* represent promising candidates for FTD risk because they have been conclusively associated with a common form of neurodegenerative dementia such as AD (Benitez et al., 2013; Guerreiro et al., 2013a). The aim of the present study was to screen for rare variants in the entire coding sequence of *TREM2* in a large sample of European patients with bv-FTD or PPA to identify associations between rare *TREM2* variants and FTD-S.

2. Methods

2.1. Patients

Spanish ($n = 539$) patients with a clinical diagnosis of FTD (according to the criteria of Neary et al., 1998) were recruited from a total of 5 study centers within the Dementia Genetics Spanish Consortium (DEGESCO) consortium. For the purposes of the present study and on the basis of all available clinical data, the patients were reclassified into clinical subgroups in accordance with the latest diagnostic criteria for bv-FTD (Rascovsky et al., 2011) and the 3 different forms of PPA (Gorno-Tempini et al., 2011). Patients with FTD and comorbid motor neuron disease (MND) were classified as having MND-FTD. Nine subjects with neuropathologically confirmed FTD with ubiquitin and TDP43 inclusions (FTD TDP43+) were also included. Cases were defined as having "unclassified FTD" (unclas-FTD) when the clinical data were insufficient to assign an accurate FTD subgroup diagnosis such as bv-FTD, one of the 3 forms of PPA, or MND-FTD.

In the German patients ($n = 63$), 47 patients were enrolled between 2001 and 2003 within the context of the German Dementia Competence Network (Kornhuber et al., 2009), that is, before the introduction of the latest diagnostic criteria. To determine the FTD subtype in these patients, the following algorithm was applied: (1) identification of neuropsychological signs/symptoms (Chare et al., 2014) from the items contained in the frontal behavioral inventory, frontal assessment battery, neuropsychiatric inventory, the spontaneous language analysis in the Aachener-Aphasie test, and the Consortium to Establish a Registry for Alzheimer's Disease; (2) bivariate coding of impairment in the neuropsychological signs/symptoms as "0" or "1" indicating absent or present, respectively; (3) grouping of the signs/symptoms into domains that can be used to diagnose the FTD subtype and generate a cut-off score; and (4) transformation of this process into an SPSS algorithm, which uses the scores to calculate a "yes" or "no" answer for the defined diagnostic

categories. The remaining 16 FTD patients were recruited in the Memory Clinic at the University of Bonn. Clinical subgroups were defined using the same procedure as with the Spanish sample.

2.2. Controls

Spanish control samples for the association study were obtained from DESGECO consortium (Table 2). The frequency of p.R47H in Spanish healthy individuals was determined using the entire Spanish control sample used by DEGESCO (Ruiz et al., 2014). To determine the allele frequency of p.T96K, 539 cognitively normal Spanish controls were selected from the DEGESCO control sample. To obtain the allele frequency of p.R62H, 127 cognitively normal Spanish controls were drawn at random from the DEGESCO control sample and genotyped.

The German controls were drawn from the AgeCoDe cohort ($n = 939$). AgeCoDe is an ongoing German multicenter prospective cohort study. The original AgeCoDe cohort was drawn at random from general practice registries in 6 German cities, and the selected individuals were contacted by letter to request participation. The main inclusion criteria were age $\geq$75 years and the absence of dementia according to the clinical judgment of the respective general practitioner. A total of 3327 AgeCoDe participants were recruited between January 2002 and November 2003 (Jessen et al., 2011; Luck et al., 2007). Each participant undergoes assessments at 18 months intervals. All assessments are performed at the participant's home by a trained study psychologist or physician. For all AgeCoDe controls included in the present study, dementia and mild cognitive dementia had been excluded at the last follow-up visit. The study was approved by the Ethics Committee of each collaborating study center. All patients and controls provided written informed consent before inclusion.

2.3. TREM2 gene sequencing and genotyping

In the FTD-S patients, genomic DNA was isolated from peripheral blood using standard procedures, and all coding exons were amplified using polymerase chain reaction. The primer sequences are presented in Supplementary Table 1. The entire coding region of *TREM2* was then screened through direct Sanger sequencing of the polymerase chain reaction products. Mutation numbering was assigned using the complementary DNA sequence of the GenBank entry NM_018965.3, in which position +1 corresponds to the A of the ATG translation initiation codon. The software packages PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) and Meta-SNP (http://snps.biofold.org/meta-snp/) were used to predict the functional and structural effects of amino acid substitutions. The meta-predictor Meta-SNP combines the output of 3 different prediction programs, that is, PhD-SNP, SIFT, and SNAP, to generate a prediction score for putative disease causing variants.

Direct genotyping of p.R62H in the Spanish controls was performed using Sequenom iPLEX (Sequenom). The p.T96K variant was genotyped in the Spanish control sample using a custom-designed TaqMan assays (Life Technologies).

In the German healthy controls, the p.R47H and p.T96K variants were genotyped using a custom-designed TaqMan assays (Life Technologies). In 1 healthy Spanish control, the p.R62H variant was present in a homozygous state. This variant was, therefore, excluded from the genotyping process in German controls.

2.4. Statistical analysis

To calculate the minor allele frequency (MAF) for controls, data from 4 different sources were combined: the published 1000 genome (http://browser.1000genomes.org/) MAFs in European population; published data by Guerreiro et al. (2013a); the MAF for each genotyped variant in the present Spanish control samples obtained from DEGESCO; and the MAF for each genotyped variant in the present German control sample. Association between case-control status and variant carrier/noncarrier status was calculated using Fisher exact test for 2×2 contingency tables. The statistical analyses were performed using the implementation in R (The R Project for Statistical Computing, www.r-project.org). The alpha level for statistical significance was set at $\leq$0.05. The genotyping data were inspected to detect deviations from Hardy-Weinberg equilibrium. All single-nucleotide polymorphisms included in the association were in Hardy-Weinberg equilibrium ($p > 0.05$).

3. Results

3.1. Rare variants in TREM2 and their associated clinical phenotype

The demographic data of the samples are summarized in Table 1.

The presence of at least 1 heterozygous rare *TREM2* variant was identified in 5.32% (32 of 602) of the FTD-S patients (Table 2). Most of these variants were located in exon 2. These variants comprised 7 previously reported rare coding variants (p.A28V, p.W44X, p.R47H, p.R62H, p.T66M, p.T96K, p.L211P) and 1 novel missense variant p.A105T (Table 2). Four of these variants, that is, p.W44X, p.R47H, p.T66M, and p.T96K, were predicted to have a probably damaging effect. The other 4 were predicted to be benign (Table 3). The most frequent variant was p.R62H (rs143332484). This was present in 19 patients, all of whom displayed heterozygous p.R62H status. Eighteen carriers were of Spanish origin, and 1 was German. Most of the p.R62H carriers were classified as bv-FTD cases (Table 2). The second most frequent rare *TREM2* variant status was the simultaneous presence of p.T96K (rs2234253) and p.L211P (rs2234256). This was observed in 6 patients, 4 Spanish (lv-PPA [$n = 1$], bv-FTD [$n = 2$], FTD-MND [$n = 1$]) and 2 German (lv-PPA [$n = 1$] and sv-PPA [$n = 1$]) patients. The third most frequent variant was p.R47H, which has previously been associated with AD. This was found in 4 patients, 3 Spanish lv-PPA patients and 1 German unclas-FTD patient (for clinical details, see

Table 1
Demographics of the FTD-S and the control samples

	bv-FTD	nfv-PPA	lv-PPA	sv-PPA	MND-FTD	FTD TDP43+	Unclas-FTD	Spanish control sample p.T96K	Spanish control sample p.R62H	German control sample
Subjects (n)	313	105	59	58	18	9	40	539	127	939
Sex (% female)	45	54	68	29	39	75	37	54	53.5	63.5
AAO (mean ± SD)	62.4 ± 14.6	64.7 ± 15.8	66.2 ± 8.6	62.0 ± 12.4	60.8 ± 14.8	75.0 ± 5.5	61.9 ± 9.1			
Age (mean ± SD)								62.4 ± 12.7	76.57 ± 4.89	86.9 ± 3.2

Key: AAO, age at onset; bv, behavioral variant; FTD, frontotemporal dementia; FTD-S, FTD subtypes; FTD TDP43+, FTD with ubiquitin and TDP43 inclusions; lv, logopenic variant; MND-FTD, FTD with comorbid motor neuron disease; nfv, nonfluent variant; PPA, primary progressive aphasia; SD, standard deviation; sv, semantic variant; unclas-FTD, unclassified FTD.

Table 2
Association between *TREM2* mutations and FTD-S

Exon	bv-FTD ($n = 299$)	nfv-PPA ($n = 96$)	lv-PPA ($n = 54$)	sv-PPA ($n = 51$)	MND-FTD ($n = 18$)	Unclas-FTD ($n = 75$)	FTD TDP43+ ($n = 9$)	Total ($n = 602$)
A28V		1 (0.95)						1 (0.17)
W44X	1 (0.32)							1 (0.17)
R47H			3 (5.01)[a]			1 (2.5)		4 (0.66)[a]
R62H	12 (3.83)	1 (0.95)	1 (1.69)[a]	2 (3.45)	1 (5.55)	1 (2.5)	1 (11.11)	19 (3.16)[a]
T66M	1 (0.32)							1 (0.17)
T96K	2 (0.64)[a]		2 (3.39)[a]	1 (1.72)[a]	1 (5.55)[a]			6 (1.0)[a]
A105T				1 (1.72)				1 (0.17)
L211P	2 (0.64)[a]		2 (3.39)[a]	1 (1.72)[a]	1 (5.55)[a]			6 (1.0)[a]
Total	16 (5.11)	2 (1.9)	5 (8.47)	4 (6.9)	2 (11.11)	2 (5.0)	1 (11.11)	32 (5.32)

Key: bv, behavioral variant; FTD, frontotemporal dementia; FTD-S, FTD subtypes; FTD TDP43+, FTD with ubiquitin and TDP43 inclusions; lv, logopenic variant; MND-FTD, FTD with comorbid motor neuron disease; nfv, nonfluent variant; PPA, primary progressive aphasia; sv, semantic variant; unclas-FTD, unclassified FTD.
[a] Patients with 2 mutations.

Supplementary Table 2). Interestingly, in one of these Spanish lv-PPA patients, the p.R47H variant was found in combination with p.R62H. Although no clear diagnosis could be established for the German unclas-FTD patient with the p.R47H variant, the available data suggested the presence of AD pathology. In fact, cerebrospinal fluid (CSF) analysis in this patient revealed reduced amyloid β_{1-42} (368.67 pg/mL, normal range >600 pg/mL), increased total tau (662 pg/mL, normal range <300 pg/mL), and increased phosphorylated-tau (93.9 pg/mL, normal range <60 pg/mL) (Supplementary Table 2). Furthermore, brain magnetic resonance imaging showed left-sided unilateral cortical brain atrophy with predominant involvement of the posterior temporal lobe and the hippocampus (Supplementary Fig. 1). With the exception of p.T66M, all remaining variants (p.A28V, p.W44X, and p.A105T) were found in single Spanish patients (Table 2).

3.2. Association between rare TREM2 variants and FTD-S

The subsequent analyses focused on the possible association of the most frequently identified variants only, that is p.R62H, p.R47H, and p.T96K. No association analysis was performed for p.L211P because this variant was present in patients carrying p.T96K, suggesting that these 2 single-nucleotide polymorphisms are in linkage disequilibrium (LD). In the Spanish sample, we did not observe association with any of the variants tested. Interestingly, the effect size and direction of the p.R47H variant were fully consistent with that reported for AD (Guerreiro et al., 2013a; Ruiz et al., 2014). The association analysis in the German sample revealed an association only for p.T96K ($p = 0.0039$, Table 4). Again, the p.R47H showed a similar effect size and allele direction as the Spanish sample. Finally, we performed an association analysis in the combined samples. For p.R62H, the association analysis using the combined results in control populations revealed no association with FTD-S ($p = 0.568$, odds ratio [OR] = 1.16 [0.63–2.05], Table 4). The hypothesis that p.R62H is not implicated in FTD-S was supported by the detection of an elderly homozygous p.R62H carrier in the Spanish control sample. For p.R47H, the combined analysis showed no association with FTD-S ($p = 0.281$, OR = 1.91 [0.46–5.93], Table 4). An association signal was only observed between p.T96K and the combined FTD-S samples ($p = 0.013$, OR = 4.23 [1.17–14.77], Table 4).

4. Discussion

To our knowledge, the present report describes the largest sequencing study of rare *TREM2* variants in FTD-S performed to date. Association was found between the rare *TREM2* variant p.T96K and FTD-S for the German sample and the combined analysis of both series. In addition, the analyses identified 2 pathogenic rare variants, that is, p.W44X and p.T66M. The p.W44X was previously reported to cause in a homozygous state polycystic lipomembranous osteodysplasia with sclerosing

Table 3
In silico analyses of *TREM2* variants identified among subjects with FTD-S

	Genomic position	Protein position (n)	SNP ID	Meta-SNP					PolyPhen2	
				RI	PhD-SNP	SIFT	SNAP	Meta-SNP	HumVar	HumDiv
Exon 2	g.41129309G>A	p.A28V (1)	rs2234252	7	Neutral 0.306	Neutral 0.307	Neutral 0.307	Neutral 0.154	Neutral 0.08	Possibly damaging 0.503
	g.41129252C>T	p.R47H (4)	rs75932628	2	Disease 0.514	Disease 0.030	Disease 0.680	Disease 0.586	Disease 0.989	Disease 1
	g.41129207C>T	p.R62H (19)	rs143332484	8	Neutral 0.216	Neutral 0.670	Neutral 0.280	Neutral 0.086	Neutral 0.008	Neutral 0.016
	g.41129195G>A	p.T66M (1)	rs201258663	2	Disease 0.544	Neutral 0.080	Disease 0.545	Disease 0.578	Disease 0.989	Disease 1
	g.41129105G>C	p.T96K (6)	rs2234253	4	Disease 0.722	Neutral 0.120	Disease 0.735	Disease 0.680	Disease 1	Disease 1
	g.41129083G>A	p.A105T (1)	Unknown	7	Neutral 0.411	Neutral 0.070	Neutral 0.315	Neutral 0.175	Neutral 0.191	Possibly damaging 0.659
Exon 4	g.41126655A>G	p.L211P (6)	rs2234256	7	Neutral 0.114	Disease 0.000	Disease 0.635	Neutral 0.136	Neutral 0.001	Neutral 0.001

Note: Meta-SNP, meta-predictor of putative disease-causing variants that combine the output of PhD-SNP, SIFT, and SNAP; outputs, value reported under the following algorithms: PhD-SNP, between 0 and 1 (>0.5 suggests predicted effect "disease"); SIFT, positive value (>0.05 suggests predicted effect "neutral"); SNAP, output normalized between 0 and 1 (>0.5 suggests predicted effect "disease"); Meta-SNP, between 0 and 1 (>0.5 suggests predicted effect "disease"); RI, reliability index between 0 and 10; PolyPhen2, between 0 and 1 (I > 0.5 suggests predicted effect "disease").
Key: FTD-S, frontotemporal dementia subtypes; disease, disease-causing variants; neutral, neutral variants; SNP, single-nucleotide polymorphism.

Table 4
Association analysis of the most frequent *TREM2* variants (p.R47H, p.R62H and p.T96K) in the present FTD-S cohort

TREM2 variant	SNP ID	Position[a]	Location	MAF FTD-S patients	All FTD-S		Spanish controls		German controls		Controls[b]		Controls recorded 1000K (EUR)[c]		All controls		MAF controls (%)	*p* Value (Fisher exact test[d])	Odds ratio (95% CI)
					C[e]	Non-C[f]	C[e]	Non-C[f]	C[e]	Non-C[f]	C[e]	Non-C[f]	C[e]	Non-C[f]	C[e]	Non-C[f]			
Combined analysis																			
R47H	rs75932628	41129252	Exon 2	0.0033	4	598	3	2166	4	917	5	1100	4	375	16	4558	0.00175	0.281	1.91 (0.46–5.93)
R62H	rs143332484	41129207	Exon 2	0.0158	19	583	4	123	0	0	31	1073	9	370	44	1566	0.0137	0.568	1.16 (0.63–2.05)
T96K	rs2234253	41129105	Exon 2	0.00498	6	596	4	526	0	939	3	1102	0	379	7	2946	0.00119	0.013	4.23 (1.17–14.77)
Spanish sample																			
R47H	rs75932628	41129252	Exon 2	0.0028	3	536	3	2166							3	2166	0.00069	0.097	4.04 (0.53–30.23)
R62H	rs143332484	41129207	Exon 2	0.0167	18	521	4	123							4	123	0.0157	1	1.06 (0.34–4.39)
T96K	rs2234253	41129105	Exon 2	0.00371	4	534	4	526							4	526	0.00378	1	0.99 (0.18–5.31)
German sample																			
R47H	rs75932628	41129252	Exon 2	0.008	1	62			4	917					4	917	0.0022	0.282	3.7 (0.07–38.02)
R62H	rs143332484	41129207	Exon 2	0.008	1	62			0	0	31	1073	9	370	40	1443	0.0135	1	0.58 (0.01–3.56)
T96K	rs2234253	41129105	Exon 2	0.0159	2	61			0	939					0	939	0	0.0039	Inf (2.82 to Inf)

Key: CI, confidence interval; FTD-S, frontotemporal dementia subtypes; MAF, minor allele frequency; MND-FTD, FTD with comorbid motor neuron disease.
[a] The variant position is indicated in terms of base pairs and is based on hg19 (GRCh37).
[b] Allele Counts are those reported in a previous study (Guerreiro et al., 2013a).
[c] http://browser.1000genomes.org/.
[d] As implemented in R (http://www.r-project.org/).
[e] Carriers.
[f] Noncarriers.

leukoencephalopathy (Paloneva et al., 2002). In the case of p.T66M, this variant was identified in a homozygous state in a Turkish patient presenting clinically with an FTD-like syndrome without bone involvement (Guerreiro et al., 2013b). In the present cohort, both variants were found each in a single subject with bv-FTD, suggesting that p.W44X and p.T66M may also contribute to the etiology of FTD-S. All pathogenic variants in the present series were located in exon 2, which encodes the IgV-set domain. This region of TREM2 is more highly conserved than other segments of the protein and may play a regulatory role in leukocyte activation (Allcock et al., 2003). Interestingly, most of the *TREM2* variants identified in previous studies of AD, PD, and FTD were also located in exon 2. These findings, together with the present data, suggest that the IgV-set domain plays a central role in the contribution of *TREM2* to neurodegeneration. However, functional studies are warranted to confirm this hypothesis.

Our findings for p.T96K suggest that it is implicated in FTD-S. However, the positive association in the combined analysis is mainly driven by the association found in the German series. Therefore, this association must be interpreted cautiously as it might be the result of a type I error because of stratification in a sample of small size, particularly the German sample. On the other hand, the p.T96K seems to be a rare variant among 939 German elder neurologically healthy individuals, whereas in 63 FTD-S patient, 2 carriers were identified. This observation supports the association found in the German sample despite the small sample size of the German FTD-S patient.

The present study did not support a major contribution of the AD-associated variant p.R47H to FTD-S. However, previous reports have found that p.R47H is 3 times more common in AD and FTD patients compared with controls (Cuyvers et al., 2014). Furthermore, Borroni et al. (2014) reported that the *TREM2* variants p.Q33X, p.R47H, p.T66M, and p.S116C were associated with the subdiagnosis of both sv-PPA and bv-FTD. However, in both studies, the observed association between FTD and p.R47H was negative. Interestingly, these studies and the present investigation showed an OR and an allele direction that are consistent with the effect seen in AD. One possible explanation is that p.R47H is not as strong a risk factor for FTD-S as it is for AD. In this case, our sample would lack the statistical power required to detect a significant association. Alternatively, the observed OR and allele direction in both the German and the Spanish FTD-S series may have been attributable to the presence in the cohort of misdiagnosed AD patients with the p.R47H variant. This possibility is given credence by the fact that some early-onset AD cases (age at onset <65 years) present with clinical features that overlap with those observed in FTD (Balasa et al., 2014). In supporting this possibility, the p.R47H variant (rs75932628) was found in 4 patients in our study, 3 of whom had a diagnosis of lv-PPA. Evaluation of the clinical, CSF biomarker, and neuroimaging data of these patients indicated possible underlying AD pathology in these lv-PPA patients (Supplementary Table 1). For the fourth German patient carrying the p.R47H, insufficient clinical data were available to assign a precise FTD-S subdiagnosis. However, abnormal CSF levels of amyloid-β42, total tau, and phosphorylated tau were present, which suggests an underlying amyloid pathology. Interestingly, several reports have suggested that lv-PPA is commonly associated with AD pathology (Warren et al., 2012). The phenotype of lv-PPA comprises specific impaired naming and sentence repetition in the absence of agrammatism (Gorno-Tempini et al., 2004). Whereas brain atrophy in nfv-PPA and sv-PPA is restricted to the frontal and temporal lobes, neurodegeneration in lv-PPA occurs principally in the left posterior temporal and inferior parietal lobes (Gorno-Tempini et al., 2004). Furthermore, postmortem and amyloid-positron emission tomography studies of lv-PPA have shown that, in contrast with other FTD-S, its neuropathologic features frequently correspond to AD. In light of these data, our findings for p.R47H suggest that this variant may lead primarily, but not exclusively, to 2 different clinical AD outcomes, that is, typical AD or lv-PPA, which share a common underlying pathology. The identification of individuals who were diagnosed with FTD but had AD is important because the misdiagnosis may result in the failure to provide appropriate medication for the patient or even potentially the prescription of inappropriate medication (Boxer et al., 2013; Hernandez et al., 2014). However, this clinical discrimination is challenging because some impaired language and executive functions are common in both FTD and AD leading to misdiagnosis (Becker et al., 1988). This is clearly seen in our series despite the stringent clinical criteria applied. Research has

tried to identify biomarkers that discriminate AD and FTD (Hernandez et al., 2014). Unfortunately, a large-scale application of these biomarkers is difficult because they are either still in an experimental phase or only available at highly specialized centers. In this regard, a simple genetic test of *TREM2* may help to define the subgroup of FTD patients for whom AD pathology may be excluded before a more definite diagnosis can be made. However, this genotype-phenotype correlation should be taken with caution because of the limited sample size. Additional studies in larger FTD-S series are now warranted to confirm this observation.

Evidence was also generated for the existence of strong LD between p.T96K and p.L211P. In support of this observation, Lattante et al. (2013) reported an FTD patient with homozygous status for both p.T96K and p.L211P. Interestingly, Cuyvers et al. (2014) identified p.T96K and p.L211P in 2 patients, respectively. However, the authors do not state whether the 2 variants were carried by the same patient. The identification of 6 patients with the simultaneous presence of 2 rare variants raises several questions concerning the role of each variant in disease presentation and severity. The clinical presentation of the simultaneous p.T96K and p.L211P carriers did not differ from that observed in patients carrying a single rare variant in *TREM2* (Supplementary Table 3). The present study identified another case with 2 rare variants in *TREM2*, that is p.R47H and p.R62H (Supplementary Table 1). This patient presented with a lv-PPA phenotype that did not differ from that of lv-PPA patients carrying a single p.R47H variant. In contrast with the situation for p.T96K and p.L211P, no evidence of LD between the 2 variants was obtained, and the lack of additional family members precluded elucidation whether or not they are in the same chromosome. Nevertheless, the available clinical data provided no support for the hypothesis that an additional pathogenic effect was conferred by the presence of 2 rare variants in the same gene. Thus, the most likely explanation is that p.L211P and p.R62H are neutral variants with no influence on disease phenotype and that their cooccurrence with the pathogenic variants p.T96K and p.R47H is coincidental. This hypothesis is supported by the fact that most prediction programs indicate that p.L211P and p.R62H are neutral. However, functional experiments are warranted to determine or exclude their pathogenic contribution.

In one of the patients with both the p.T96K and p.L211P variants, the pathologic G4C2 repeat expansion in C9ORF72 was also observed (Supplementary Table 3). Previous authors have described carriers of mutations in 2 different FTD genes (Cuyvers et al., 2014; van Blitterswijk et al., 2013). For example, in FTD patients carrying rare variants in *TREM2*, a second pathologic mutation was also identified in either *C9ORF72* or *VCP* (p.R159H) (Cuyvers et al., 2014). In one study, the phenotype of these rare cases appeared to have an earlier age of disease onset, although statistical comparisons were not possible because of the small sample size (van Blitterswijk et al., 2013). Thus, heterozygous *TREM2* variants may modify the phenotype caused by a second (pathogenic) mutation (Pastor, 2013). This may result, for example, in a different dementia phenotype compared with that observed in single-mutation carriers. However, besides the apparent earlier disease onset, the bv-FTD phenotype was unaffected by the simultaneous presence of 2 mutations in different genes. Similarly, our patient presented also pure bv-FTD without additional symptoms. Hence, no definite conclusions can be drawn concerning the pathogenic contribution of rare *TREM2* variants to the phenotype observed in these patients. To address this issue, further studies of larger samples are required.

The present study had several limitations. First, despite being the largest systematically screened FTD-S series reported to date, the size of the sample was small given the rarity of *TREM2* mutations. This, together with the limited sample size for each FTD subdiagnosis, prevented more detailed phenotype-genotype analyses. Second, several studies have reported that rare variants display different MAF in healthy individuals from different populations. Although the MAF for controls was representative of different European populations, the proportion of individuals from the Spanish population in our control sample was small, with the exception of p.R47H carriers (Ruiz et al., 2014). Thus, the frequency observed in our control sample may not represent the MAF observed in the Spanish population, which would in turn influence the strength of our association with p.T96K. Furthermore, the lack of *TREM2* sequencing data from controls precluded the performance of a burden test.

In summary, the present results support the hypothesis that rare *TREM2* variants are implicated in the etiology of FTD-S, in particular p.T96K. Furthermore, our data suggest that the p.R47H variant might be a causal factor for lv-PPA, an FTD-S that is possibly closely related to AD. These data suggest that replication and functional studies in further FTD-S cohorts are warranted to elucidate genotype-phenotype correlations in *TREM2* mutation carriers. These studies should involve analysis of CSF biomarkers and neuropathologic confirmation of FTD-S.

Disclosure statement

The authors have no actual or potential conflicts of interest.

Acknowledgements

We thank all participants for their contribution to this project. We are indebted to Mrs Trinitat Port-Carbó and her family for their support to the Fundació ACE research programs. The work described in the present publication was performed within the context of the German Research Network on Dementia (KND) and the German Research Network on Degenerative Dementia (KNDD), which are funded by the German Federal Ministry of Education and Research (grants KND:01GI0102, 01GI0420, 01GI0422, 01GI0423, 01GI0429, 01GI0431, 01GI0433, 01GI0434; grants KNDD: 01GI1007A, 01GI0710, 01GI0711, 01GI0712, 01GI0713, 01GI0714, 01GI0715, 01GI0716, 01ET1006B). This work was supported by grants from the Department of Health of the Government of Navarra, Spain to PP (references 13085 and 3/2008). AR is supported by grant PI13/02434 (Instituto de Salud Carlos III, Ministerio de Economía y Competitividad, Spain). This study was supported in part by the Spanish Ministry of Economy and Competitiveness, Instituto de Salud Carlos III cofunded by FEDER (Fondo Europeo de Desarrollo Regional) (PI11/00234). This study was partially supported by grants from Instituto de Salud Carlos III (PI12/01311).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.neurobiolaging.2014.06.018.

References

Allcock, R.J., Barrow, A.D., Forbes, S., Beck, S., Trowsdale, J., 2003. The human TREM gene cluster at 6p21.1 encodes both activating and inhibitory single IgV domain receptors and includes NKp44. Eur. J. Immunol. 33, 567–577.

Balasa, M., Sánchez-Valle, R., Antonell, A., Bosch, B., Olives, J., Rami, L., Castellví, M., Molinuevo, J.L., Lladó, A., 2014. Usefulness of biomarkers in the diagnosis and prognosis of early-onset cognitive impairment. J. Alzheimers Dis 40, 919–927.

Becker, J.T., Huff, F.J., Nebes, R.D., Holland, A., Boller, F., 1988. Neuropsychological function in Alzheimer's disease. Pattern of impairment and rates of progression. Arch. Neurol. 45, 263–268.

Benitez, B.A., Cooper, B., Pastor, P., Jin, S.C., Lorenzo, E., Cervantes, S., Cruchaga, C., 2013. TREM2 is associated with the risk of Alzheimer's disease in Spanish population. Neurobiol. Aging 34, 1711.e15–1711.e17.

Borroni, B., Ferrari, F., Galimberti, D., Nacmias, B., Barone, C., Bagnoli, S., Fenoglio, C., Piaceri, I., Archetti, S., Bonvicini, C., Gennarelli, M., Turla, M., Scarpini, E., Sorbi, S., Padovani, A., 2014. Heterozygous TREM2 mutations in frontotemporal dementia. Neurobiol. Aging 35, 934.e7–934.e10.

Boxer, A.L., Knopman, D.S., Kaufer, D.I., Grossman, M., Onyike, C., Graf-Radford, N., Mendez, M., Kerwin, D., Lerner, A., Wu, C.K., Koestler, M., Shapira, J., Sullivan, K., Klepac, K., Lipowski, K., Ullah, J., Fields, S., Kramer, J.H., Merrilees, J., Neuhaus, J., Mesulam, M.M., Miller, B.L., 2013. Memantine in patients with frontotemporal lobar degeneration: a multicentre, randomised, double-blind, placebo-controlled trial. Lancet Neurol. 12, 149–156.

Cairns, N.J., Bigio, E.H., Mackenzie, I.R., Neumann, M., Lee, V.M., Hatanpaa, K.J., White III, C.L., Schneider, J.A., Grinberg, L.T., Halliday, G., Duyckaerts, C., Lowe, J.S., Holm, I.E., Tolnay, M., Okamoto, K., Yokoo, H., Murayama, S., Woulfe, J., Munoz, D.G., Dickson, D.W., Ince, P.G., Trojanowski, J.Q., Mann, D.M., 2007. Neuropathologic diagnostic and nosologic criteria for frontotemporal lobar degeneration: consensus of the Consortium for Frontotemporal Lobar Degeneration. Acta Neuropathol. 114, 5–22.

Chare, L., Hodges, J.R., Leyton, C.E., McGinley, C., Tan, R.H., Kril, J.J., Halliday, G.M., 2014. New criteria for frontotemporal dementia syndromes: Clinical and pathological diagnostic implications. J. Neurol. Neurosurg. Psychiatry. http://dx.doi.org/10.1136/jnnp-2013-306948.

Cruts, M., Gijselinck, I., van der Zee, J., Engelborghs, S., Wils, H., Pirici, D., Rademakers, R., Vandenberghe, R., Dermaut, B., Martin, J.J., van Duijn, C., Peeters, K., Sciot, R., Santens, P., De Pooter, T., Mattheijssens, M., Van den Broeck, M., Cuijt, I., Vennekens, K., De Deyn, P.P., Kumar-Singh, S., Van Broeckhoven, C., 2006. Null mutations in progranulin cause ubiquitin-positive frontotemporal dementia linked to chromosome 17q21. Nature 442, 920–924.

Cuyvers, E., Bettens, K., Philtjens, S., Van Langenhove, T., Gijselinck, I., van der Zee, J., Engelborghs, S., Vandenbulcke, M., Van Dongen, J., Geerts, N., Maes, G., Mattheijssens, M., Peeters, K., Cras, P., Vandenberghe, R., De Deyn, P.P., Van Broeckhoven, C., Cruts, M., Sleegers, K., 2014. Investigating the role of rare heterozygous TREM2 variants in Alzheimer's disease and frontotemporal dementia. Neurobiol. Aging 35, 726.e11–726.e19.

DeJesus-Hernandez, M., Mackenzie, I.R., Boeve, B.F., Boxer, A.L., Baker, M., Rutherford, N.J., Nicholson, A.M., Finch, N.A., Flynn, H., Adamson, J., Kouri, N., Wojtas, A., Sengdy, P., Hsiung, G.Y., Karydas, A., Seeley, W.W., Josephs, K.A., Coppola, G., Geschwind, D.H., Wszolek, Z.K., Feldman, H., Knopman, D.S., Petersen, R.C., Miller, B.L., Dickson, D.W., Boylan, K.B., Graff-Radford, N.R., Rademakers, R., 2011. Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. Neuron 72, 245–256.

Dobson-Stone, C., Hallupp, M., Loy, C.T., Thompson, E.M., Haan, E., Sue, C.M., Panegyres, P.K., Razquin, C., Seijo-Martinez, M., Rene, R., Gascon, J., Campdelacreu, J., Schmoll, B., Volk, A.E., Brooks, W.S., Schofield, P.R., Pastor, P., Kwok, J.B., 2013. C9ORF72 repeat expansion in Australian and Spanish frontotemporal dementia patients. PLoS One 8, e56899.

Gorno-Tempini, M.L., Murray, R.C., Rankin, K.P., Weiner, M.W., Miller, B.L., 2004. Clinical, cognitive and anatomical evolution from nonfluent progressive aphasia to corticobasal syndrome: a case report. Neurocase 10, 426–436.

Gorno-Tempini, M.L., Hillis, A.E., Weintraub, S., Kertesz, A., Mendez, M., Cappa, S.F., Ogar, J.M., Rohrer, J.D., Black, S., Boeve, B.F., Manes, F., Dronkers, N.F., Vandenberghe, R., Rascovsky, K., Patterson, K., Miller, B.L., Knopman, D.S., Hodges, J.R., Mesulam, M.M., Grossman, M., 2011. Classification of primary progressive aphasia and its variants. Neurology 76, 1006–1014.

Guerreiro, R., Wojtas, A., Bras, J., Carrasquillo, M., Rogaeva, E., Majounie, E., Cruchaga, C., Sassi, C., Kauwe, J.S., Younkin, S., Hazrati, L., Collinge, J., Pocock, J., Lashley, T., Williams, J., Lambert, J.C., Amouyel, P., Goate, A., Rademakers, R., Morgan, K., Powell, J., St George-Hyslop, P., Singleton, A., Hardy, J., 2013a. TREM2 variants in Alzheimer's disease. New Engl. J. Med. 368, 117–127.

Guerreiro, R.J., Lohmann, E., Bras, J.M., Gibbs, J.R., Rohrer, J.D., Gurunlian, N., Dursun, B., Bilgic, B., Hanagasi, H., Gurvit, H., Emre, M., Singleton, A., Hardy, J., 2013b. Using exome sequencing to reveal mutations in TREM2 presenting as a frontotemporal dementia-like syndrome without bone involvement. JAMA Neurol. 70, 78–84.

Hernandez, I., Mauleon, A., Rosense-Roca, M., Alegret, M., Vinyes, G., Espinosa, A., Sotolongo-Grau, O., Becker, J.T., Valero, S., Tarraga, L., Lopez, O.L., Ruiz, A., Boada, M., 2014. Identification of misdiagnosed fronto-temporal dementia using APOE genotype and phenotype-genotype correlation analyses. Curr. Alzheimer. Res. 11, 182–191.

Hutton, M., Lendon, C.L., Rizzu, P., Baker, M., Froelich, S., Houlden, H., Pickering-Brown, S., Chakraverty, S., Isaacs, A., Grover, A., Hackett, J., Adamson, J., Lincoln, S., Dickson, D., Davies, P., Petersen, R.C., Stevens, M., de Graaff, E., Wauters, E., van Baren, J., Hillebrand, M., Joosse, M., Kwon, J.M., Nowotny, P., Che, L.K., Norton, J., Morris, J.C., Reed, L.A., Trojanowski, J., Basun, H., Lannfelt, L., Neystat, M., Fahn, S., Dark, F., Tannenberg, T., Dodd, P.R., Hayward, N., Kwok, J.B., Schofield, P.R., Andreadis, A., Snowden, J., Craufurd, D., Neary, D., Owen, F., Oostra, B.A., Hardy, J., Goate, A., van Swieten, J., Mann, D., Lynch, T., Heutink, P., 1998. Association of missense and 5'-splice-site mutations in tau with the inherited dementia FTDP-17. Nature 393, 702–705.

Jessen, F., Wiese, B., Bickel, H., Eifflander-Gorfer, S., Fuchs, A., Kaduszkiewicz, H., Kohler, M., Luck, T., Mosch, E., Pentzek, M., Riedel-Heller, S.G., Wagner, M., Weyerer, S., Maier, W., van den Bussche, H., 2011. Prediction of dementia in primary care patients. PLoS One 6, e16852.

Kornhuber, J., Schmidtke, K., Frolich, L., Perneczky, R., Wolf, S., Hampel, H., Jessen, F., Heuser, I., Peters, O., Weih, M., Jahn, H., Luckhaus, C., Hull, M., Gertz, H.J., Schroder, J., Pantel, J., Rienhoff, O., Seuchter, S.A., Ruther, E., Henn, F., Maier, W., Wiltfang, J., 2009. Early and differential diagnosis of dementia and mild cognitive impairment: design and cohort baseline characteristics of the German Dementia Competence Network. Dement. Geriatr. Cogn. Disord. 27, 404–417.

Lattante, S., Le Ber, I., Camuzat, A., Dayan, S., Godard, C., Van Bortel, I., De Septenville, A., Ciura, S., Brice, A., Kabashi, E., 2013. TREM2 mutations are rare in a French cohort of patients with frontotemporal dementia. Neurobiol. Aging 34, 2443.e1–2443.e2.

Luck, T., Riedel-Heller, S.G., Kaduszkiewicz, H., Bickel, H., Jessen, F., Pentzek, M., Wiese, B., Koelsch, H., van den Bussche, H., Abholz, H.H., Moesch, E., Gorfer, S., Angermeyer, M.C., Maier, W., Weyerer, S., 2007. Mild cognitive impairment in general practice: age-specific prevalence and correlate results from the German study on ageing, cognition and dementia in primary care patients (AgeCoDe). Dement. Geriatr. Cogn. Disord. 24, 307–316.

McKhann, G.M., Albert, M.S., Grossman, M., Miller, B., Dickson, D., Trojanowski, J.Q., 2001. Clinical and pathological diagnosis of frontotemporal dementia: report of the Work Group on Frontotemporal Dementia and Pick's Disease. Arch. Neurol. 58, 1803–1809.

Neary, D., Snowden, J.S., Gustafson, L., Passant, U., Stuss, D., Black, S., Freedman, M., Kertesz, A., Robert, P.H., Albert, M., Boone, K., Miller, B.L., Cummings, J., Benson, D.F., 1998. Frontotemporal lobar degeneration: a consensus on clinical diagnostic criteria. Neurology 51, 1546–1554.

Paloneva, J., Manninen, T., Christman, G., Hovanes, K., Mandelin, J., Adolfsson, R., Bianchin, M., Bird, T., Miranda, R., Salmaggi, A., Tranebjaerg, L., Konttinen, Y., Peltonen, L., 2002. Mutations in two genes encoding different subunits of a receptor signaling complex result in an identical disease phenotype. Am. J. Hum. Genet. 71, 656–662.

Pastor, P., 2013. Comment: double mutants of frontotemporal dementia genes—simple co-occurrence? Neurology 81, 1338.

Pastor, P., Pastor, E., Carnero, C., Vela, R., Garcia, T., Amer, G., Tolosa, E., Oliva, R., 2001. Familial atypical progressive supranuclear palsy associated with homozygosity for the delN296 mutation in the tau gene. Ann. Neurol. 49, 263–267.

Rascovsky, K., Hodges, J.R., Knopman, D., Mendez, M.F., Kramer, J.H., Neuhaus, J., van Swieten, J.C., Seelaar, H., Dopper, E.G., Onyike, C.U., Hillis, A.E., Josephs, K.A., Boeve, B.F., Kertesz, A., Seeley, W.W., Rankin, K.P., Johnson, J.K., Gorno-Tempini, M.L., Rosen, H., Prioleau-Latham, C.E., Lee, A., Kipps, C.M., Lillo, P., Piguet, O., Rohrer, J.D., Rossor, M.N., Warren, J.D., Fox, N.C., Galasko, D., Salmon, D.P., Black, S.E., Mesulam, M., Weintraub, S., Dickerson, B.C., Diehl-Schmid, J., Pasquier, F., Deramecourt, V., Lebert, F., Pijnenburg, Y., Chow, T.W., Manes, F., Grafman, J., Cappa, S.F., Freedman, M., Grossman, M., Miller, B.L., 2011. Sensitivity of revised diagnostic criteria for the behavioural variant of frontotemporal dementia. Brain 134 (Pt 9), 2456–2477.

Renton, A.E., Majounie, E., Waite, A., Simon-Sanchez, J., Rollinson, S., Gibbs, J.R., Schymick, J.C., Laaksovirta, H., van Swieten, J.C., Myllykangas, L., Kalimo, H., Paetau, A., Abramzon, Y., Remes, A.M., Kaganovich, A., Scholz, S.W., Duckworth, J., Ding, J., Harmer, D.W., Hernandez, D.G., Johnson, J.O., Mok, K., Ryten, M., Trabzuni, D., Guerreiro, R.J., Orrell, R.W., Neal, J., Murray, A., Pearson, J., Jansen, I.E., Sondervan, D., Seelaar, H., Blake, D., Young, K., Halliwell, N., Callister, J.B., Toulson, G., Richardson, A., Gerhard, A., Snowden, J., Mann, D., Neary, D., Nalls, M.A., Peuralinna, T., Jansson, L., Isoviita, V.M., Kaivorinne, A.L., Holtta-Vuori, M., Ikonen, E., Sulkava, R., Benatar, M., Wuu, J., Chio, A., Restagno, G., Borghero, G., Sabatelli, M., Heckerman, D., Rogaeva, E., Zinman, L., Rothstein, J.D., Sendtner, M., Drepper, C., Eichler, E.E., Alkan, C., Abdullaev, Z., Pack, S.D., Dutra, A., Pak, E., Hardy, J., Singleton, A., Williams, N.M., Heutink, P., Pickering-Brown, S., Morris, H.R., Tienari, P.J., Traynor, B.J., 2011. A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. Neuron 72, 257–268.

Ruiz, A., Dols-Icardo, O., Bullido, M.J., Pastor, P., Rodriguez-Rodriguez, E., Lopez de Munain, A., de Pancorbo, M.M., Perez-Tur, J., Alvarez, V., Antonell, A., Lopez-Arrieta, J., Hernandez, I., Tarraga, L., Boada, M., Lleo, A., Blesa, R., Frank-Garcia, A., Sastre, I., Razquin, C., Ortega-Cubero, S., Lorenzo, E., Sanchez-Juan, P., Combarros, O., Moreno, F., Gorostidi, A., Elcoroaristizabal, X., Baquero, M., Coto, E., Sanchez-Valle, R., Clarimon, J., 2014. Assessing the role of the TREM2 p.R47H variant as a risk factor for Alzheimer's disease and frontotemporal dementia. Neurobiol. Aging 35, 444.e1–444.e2.

van Blitterswijk, M., Baker, M.C., DeJesus-Hernandez, M., Ghidoni, R., Benussi, L., Finger, E., Hsiung, G.Y., Kelley, B.J., Murray, M.E., Rutherford, N.J., Brown, P.E., Ravenscroft, T., Mullen, B., Ash, P.E., Bieniek, K.F., Hatanpaa, K.J., Karydas, A., Wood, E.M., Coppola, G., Bigio, E.H., Lippa, C., Strong, M.J., Beach, T.G., Knopman, D.S., Huey, E.D., Mesulam, M., Bird, T., White 3rd, C.L., Kertesz, A., Geschwind, D.H., Van Deerlin, V.M., Petersen, R.C., Binetti, G., Miller, B.L., Petrucelli, L., Wszolek, Z.K., Boylan, K.B., Graff-Radford, N.R., Mackenzie, I.R., Boeve, B.F., Dickson, D.W., Rademakers, R., 2013. C9ORF72 repeat expansions in cases with previously identified pathogenic mutations. Neurology 81, 1332–1341.

Warren, J.D., Fletcher, P.D., Golden, H.L., 2012. The paradox of syndromic diversity in Alzheimer disease. Nat. Rev. Neurol. 8, 451–464.

Journal of Alzheimer's Disease 5 (2003) 445–454
IOS Press

Ubiquitin-negative mini-pick-like bodies in the dentate gyrus in p301l tauopathy

Isidro Ferrer[a,b,*], Isabel Hernández[c], Berta Puig[a], María Jesús Rey[b], Mario Ezquerra[b,d], Eduardo Tolosa[b,d] and Merce Boada[c]
[a]*Institut de Neuropatologia, Servei Anatomia Patològica, Hospital de Bellvitge, Universitat de Barcelona, Hospitalet de Llobregat, Spain*
[b]*Banc de Teixits Neurològics, Barcelona, Spain*
[c]*Fundacio ACE, Institut Catala de Neurociències Aplicades, Barcelona, Spain*
[d]*Servei de Neurologòa, Hospital Clinic, Barcelona, Spain*

Abstract. Neuropathological and biochemical findings are reported in a patient who had suffered from frontotemporal dementia associated with a P310L mutation in the *tau* gene and included in the H1 haplotype.
Tau accumulation, as revealed with phospho-specific anti-*tau* antibodies Thr181, Ser199, Ser202, Ser214, Ser262, Ser396, Ser422 and AT8 (Ser202 and Thr205), was found in neurons with pre-tangles, and astrocytes and oligodendrocytes through the brain. The most characteristic feature was *tau* immunoreactivity decorating the perinuclear region and small cytoplasmic aggregates designed as mini-Pick-like bodies, mainly in the dentate gyrus. Inclusions were not stained with anti-ubiquitin antibodies and did not recruit tubulins. Tau accumulation in individual cells was associated with increased expression of kinases linked with *tau* phosphorylation, mainly active (phosphorylated) stress kinases SAPK/JNK and p38 (SAPK/JNK-P and p38-P). Phosphorylated GSK-3β at Ser9 (GSK-3β-P), that inactivates the kinase, was particularly abundant in mini-Pick-like bodies, thus suggesting alternative roles of GSK-3 probably involved in cell survival. Western blots of sarkosyl-insoluble fractions revealed a double band pattern of phospho-*tau* of 68/66 kDa and 64 kDa in the hippocampus and white matter in the P310L mutation. Sarkosyl-insoluble fractions of the hippocampus were enriched in p38-P and GSK-3β-P in Alzheimer's disease (AD) cases, processed in parallel for comparative purposes, but not in the P310L mutation. In addition, no bands of high molecular weight were found in P310L in contrast with AD in these fractions. These findings indicate that the major sites of *tau* phosphorylation, and the expression of kinases involved in *tau* phosphorylation are active in P310L mutation as in AD and other tauopathies. Yet the P310L mutation has particular phospho-*tau* inclusions that are not tag with ubiquitin and appear to be rather soluble when compared with AD.

1. Introduction

Tauopathies are neurodegenerative diseases characterized by the abnormal hyper-phosphorylation and deposition of *tau* in neurons and glial cells. Alzheimer's disease (AD), Pick's disease (PiD), progressive supranuclear palsy, corticobasal degeneration and argyrophilic grain disease (AGD) are common tauopathies [7,27,38–40]. In addition, the term frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17) designates inherited tauopathies associated with mutations in the *tau* gene [7,16,23, 27]. Tauopathies have been reproduced in transgenic mice [17,22,28].

The P301L mutation is a common cause of familial tauopathy [1,6,26,31,33,36,37]. Like other *tau* mutations in the binding region encoded by exon 10, the P301L mutation greatly reduces the capacity to promote microtubule assembly but results in abnormal fibrillar aggregation [3,20,42]. Frontotemporal atrophy with ballooned neurons in the frontal cortex, widespread *tau*-immunoreactive neurons and glial cells, and a pattern of two bands of 66/68 kDa and

*Corresponding author: Prof. I. Ferrer, Institut de Neuropatologia, Servei Anatomia Patològica, Hospital Universitari de Bellvitge, carrer Feixa Llarga sn; 08907 Hospitalet de Llobregat, Spain. E-mail: 8082ifa@comb.es or iferrer@csub.scs.es.

ISSN 1387-2877/03/$8.00

64 kDa on Western blots have been observed in previous cases [1,31,37]. In addition, some structural aspects of *tau* phosphorylation in neurons and glial cells associated with the P301L mutation differ from one case to another. Thus, neurofibrillary tangles, and astrocytic plaques and tuft-like astrocytes appear to be common in some cases but are rare in others [1,31,16]. This indicates that, in addition to the common mutation, other factors, including the haplotype, may influence the phenotype of the mutation [33]. Previous studies have shown the P301L mutation in the H2 haplotype [41]. Interestingly, the presence of ubiquitin-negative, mutated *tau*-positive perinuclear reinforcements and small cytoplasmic aggregates reminiscent of mini-Pick bodies in neurons of selected areas, mainly granule cells of the dentate gyrus, has been recently emphasized in two patients with frontotemporal dementia bearing the P301L mutation [1]. The P301L mutation has been generated in two transgenic lines in mice [19,29,30]. In one line, Pick-like bodies are found in select brain regions [29].

The present study focuses on the *tau* deposits in neurons in a new case of P301L tauopathy with particular attention to *tau* phosphorylation and aggregation, and to the local expression of kinases involved in *tau* phosphorylation.

2. Material and methods

2.1. Case report

The proband was a man who had suffered from disturbances in social behavior starting at the age of 45 years, followed by intellectual impairment, language disorder, difficulty in calculation but with preservation of orientation and memory. The MRI carried out at the age of 49 showed frontotemporal atrophy. The course of the disease was progressive with apathy, irritability, apraxia and epilepsy, together with marked loss of language abilities. The patient died at the age of 52 years. His mother was affected by a similar disease; no neurological examination and post-mortem study was available.

The post-mortem delay between death and tissue processing was 3 hours.

2.2. Genetic study

Exons of the *tau* gene were amplified by using primers derived from $3'$ and $5'$ intronic sequences and polymerase chain reaction (PCR) conditions previously described [21] and analyzed through single strand conformation polymorphism (SSCP). After, electrophoresis, the gel was silver stained as described [4]. The SSCP analysis of exon 10 indicated the presence of an abnormal pattern in the proband when compared with controls. The polymerase chain reaction (PCR) product corresponding to the sample with the abnormal SSCP pattern was sequenced using the ABIPRISM Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin–Elmer, Foster City, CA, USA) following the manufacturer's protocols and an automatic sequencer (ABI PRISM model 377). The exon 10 sequence revealed the presence of the P301L mutation in heterozygosis in both, the sense and the complementary strand. Tau haplotype H1/H1 was determined genotyping one of the polymorphism previously described [10].

2.3. Neuropathological study

The neuropathological examination was carried out in formalin-fixed tissue for no less than three weeks; the tissue was then embedded in paraffin. De-waxed sections, 5 μm thick, were stained with haematoxylin and eosin, and luxol fast blue-Klüver Barrera or processed for immunohistochemistry following the streptavidin LSAB method (Dako, Dakopats, Barcelona, Spain). After incubation with methanol and normal serum, the sections were incubated with one of the primary antibodies at 4°C overnight. Antibodies to phosphorylated neurofilaments of 170 kD or 200 kD (clones BF10 and RT97, Boehringer-Mannheim, Barcelona, Spain) were used at dilutions of 1:100 and 1:50, respectively. Antibodies to glial fibrillary acidic protein (GFAP, Dako), βA4-amyloid (Boehringer-Mannheim), and ubiquitin (Dako) were used at dilutions of 1:250, 1:5, and 1:200, respectively. Antibodies to α-synuclein (Dako) were used at a dilution of 1:100. Antibodies to pan-*tau* (Sigma, Madrid, Spain) were used at a dilution of 1:10. In addition, the following phospho-specific *tau* rabbit polyclonal antibodies were used: Thr181, Ser199, Ser202, Ser214, Ser231, Ser262, Ser396 and Ser422 (all of them from Calbiochem, VWR, Barcelona, Spain). The antibodies were used at a dilution of 1:100, excepting anti-phospho-tauThr181, which was used at a dilution of 1:250. The monoclonal antibody AT8 (Dr. J. Avila), that recognizes *tau* protein phosphorylated at both Ser202 and Thr205 [17] was used at a dilution of 1:200. Monoclonal antibodies to α-tubulin (Neomarkers, Molecular Probes, Leiden, The Netherlands) and rabbit polyclonal antibodies

to β2-tubulin (Dr. J. Avila) were used at dilutions of 1:100 and 1:500, respectively. Following incubation with the primary antibody, the sections were incubated with LSAB for 1 h at room temperature. The peroxidase reaction was visualized with 0.05% diaminobenzidine and 0.01% hydrogen peroxide. Sections were counterstained with haematoxylin. Sections processed for phospho-*tau* immunohistochemistry were boiled in citrate buffer prior to the incubation with the primary antibody. Sections processed for βA4-amyloid and α-synuclein were pre-treated with 95% formic acid.

2.4. Kinase immunohistochemistry

Sections were processed with the streptavidin LSAB method, as previously. Sections were boiled in citrate buffer and then processed for immunohistochemistry. The anti-MAP kinase phospho-specific (MAPK/ERK-P) rabbit polyclonal antibody (Calbiochem) is raised against a synthetic phospho-tyrosine peptide corresponding to residues 196–209 of human p44 MAP kinase. The antibody detects phosphorylated Tyr204 of p44 and p42 MAP kinases (phospho-ERK1 and ERK2). The purified phospho-p38 MAP kinase (Thr180/Tyr182) (p38-P) rabbit polyclonal antibody (Cell Signaling, Izasa, Barcelona, Spain) detects p38 MAP kinase only when activated by dual phosphorylation at Thr180 and Tyr182. The purified rabbit polyclonal phospho-SAPK/JNK (Thr183/Tyr185) antibody (SAPK/JNK-P) (Cell Signaling) is produced against a synthetic phospho-Thr183/Tyr185 peptide corresponding to the residues of human SAPK/JNK. The antibody detects SAPK/JNK only when activated by phosphorylation at Thr183/Tyr185. The antibodies to MAPK/ERK-P and SAPK/JNK-P were used at a dilution of 1:100. The antibody to p38-P was used at a dilution of 1:200. The anti-GSK-3α/β monoclonal antibody (StressGen, Bionova, Madrid, Spain) reacts with 51 and 47 kDa proteins corresponding to the specific molecular weight of GSK-3 α and GSK-3β. The antibody was used at a dilution of 1:100. The anti-phospho-specific GSK-3βSer9 antibody (Oncogene, Bionova, Madrid, Spain) is a rabbit polyclonal IgG antibody specific for the Ser9 phosphorylated form of glycogen synthase kinase-3β. The antibody was used at a dilution of 1:100.

2.5. Biochemical study

For gel electrophoresis and Western blotting, fresh samples of the P301L mutation were immediately obtained at autopsy, frozen in liquid nitrogen, and stored at -80°C until use. For comparative purposes, fresh hippocampal samples of four patients with Alzheimer's disease stage V of Braak and Braak, with similar postmortem delays (between 2 and 5 h), were processed in parallel.

Fresh samples from the hippocampus and white matter (about 5 g) were homogenized in a glass tissue grinder in 10 vol (w/v) of cold suspension buffer consisting of 10 mM Tris-HCl (pH=7.4), 0.8 M NaCl, 1 mM EGTA, 10% sucrose, 0.1 mM phenylmethylsulfonyl fluoride, 2 μg/ml aprotinin, 10 μg/ml leupeptin and 5 μg/ml pepstatin. The homogenates were first centrifuged at 20,000 $\times$ g, and the supernatant (S1) was retained. The pellet (P1) was re-homogenized in 5 vol of homogenization buffer and re-centrifuged. The two supernatants (S1+S2) were then mixed and incubated with N-lauroylsarcosynate 1% for 1 h at room temperature while shaking. Samples were then centrifuged for 1 h at 100,000 $\times$ g in a Ti 70 Beckman rotor. Sarkosyl-insoluble pellets (P3) were resuspended (0.2 ml per g of starting material) in 50 mM Tris-HCl (pH=7.4). Protein concentrations were determined by the BCA method and 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was run using a maxi-protean system (Bio-Rad, Madrid, Spain). 100–200 μg of protein was loaded in each lane with loading buffer containing 0.125 M Tris (pH=6.8), 20% glycerol, 10% mercaptoethanol, 4% SDS and 0.002% bromophenol blue. Samples were heated at 95°C for 5 min prior to gel loading. Total homogenates and fractions enriched with abnormal filaments were run in parallel. The proteins were then transferred to nitrocellulose membranes (Amersham) using an electrophoretic chamber system (Trans-Blot Electrophoretic Transfer Cell, Bio-Rad). Non-specific binding sites were blocked with Tris-buffered saline solution pH=7.4 with 0.1% Tween-20 (TBST) containing 5% skimmed milk for 30 min, and incubated with one of the primary antibodies for 1 h at room temperature. Control of protein content in each lane was carried out by the staining of selected gels with Coomassie blue and of the membranes with Ponceau (Sigma). The rabbit polyclonal antibody to phospho-tauThr181 was diluted 1:250. The rabbit polyclonal antibodies anti-phospho-tauSer262 andSer422 (all from Calbiochem) were used at a dilution of 1:1500. The p38-P antibody (Cell Signaling) was used at a dilution of 1:200. The anti-GSK-3βSer9 antibody (Oncogene) was used at a dilution of 1:100. After washing, the membranes were incubated with the secondary antibody labeled with

horseradish peroxidase (Dako) diluted 1:1000 for 1 h at room temperature, washed again, and developed with the chemiluminescence ECL Western Blotting system (Amersham, Barcelona, Spain). Membranes were then exposed to autoradiographic films (Hyperfilm ECL, Amersham).

3. Results

3.1. General neuropathological findings

The macroscopical examination revealed moderate atrophy of the brain (1300 g) predominating in the frontal and temporal lobes, and striatum and slight atrophy of the thalamus. The cerebellum and brain stem were normal with the exception of slight pallor in the substantia nigra.

The microscopic study showed severe neuron loss and increased numbers of astrocytes in the cerebral cortex, and spongiosis in the upper layers predominating in the frontal and temporal lobes (Fig. 1 A). Ballooned neurons filled with phosphorylated neurofilaments and containing αB-crystallin were common in the frontal cortex (Fig. 1 B). The hippocampus and the dentate gyrus were best preserved in haematoxylin and eosin-stained sections, although pale cytoplasmic inclusions were seen in a few granule cells (Fig. 1 C). Slight neuron loss and astrocytic gliosis were present in the striatum, amygdala and substantia nigra. Slight myelin pallor, together with astrocytic gliosis, was found in the centrum semi-ovale. No α-synuclein inclusions and βA4-amyloid deposits were found.

Sections stained with anti-*tau* antibodies disclosed the presence of *tau* accumulation in neurons in the upper and inner layers of the cerebral neocortex (Fig. 2 A), entorhinal cortex (Fig. 2 B), subiculum and cellular layer of the hippocampus (Fig. 2 C), thalamus, subthalamus, striatum (Fig. 2 D), dentate gyrus (Fig. 2 E), amygdala and Meynert nucleus (Fig. 2 E), among other gray nuclei. Interestingly, *tau* deposition in the cerebral cortex displayed a peculiar pattern involving layer II/III and layer V neurons. The majority of neurons were pre-tangles, whereas neurofibrillary tangles were very rare. The most characteristic finding was *tau*-immunoreactivity decorating the perinuclear region and, particularly, small cytoplasmic aggregates resembling mini-Pick-like bodies. Mini-Pick-like bodies were most common in the dentate gyrus (Fig. 2 E) but were seldom encountered in the cerebral cortex (Fig. 2 B) and deep cerebral nuclei (Fig. 2 E). A few *tau*-immunoreacte substantia nigra, locus ceruleus, periaqueductal and periventricular nuclei, and reticular formation of the brain stem. In addition to neurons, *tau*-immunoreactive astrocytes were found in the cerebral cortex (Fig. 2 G and H). Astrocytic plaques were seen in the frontal cortex but not in the hippocampus and dentate gyrus. Tuft-like astrocytes were absent. Oligodendroglial inclusions, some of them reminiscent of coiled bodies, were abundant in the white matter (Fig. 2 I).

Tau-immunoreactive inclusions in neurons and glial cells were examined with antibodies to phospho-*tau*. Similar findings were found with the phospho-specific anti-*tau* antibodies AT8, and Thr181, Ser199, Ser202, Ser214, Ser262, Ser396 and Ser422. Pre-tangles, perinuclear halos and cytoplasmic neuronal aggregations designed as mini-Pick-like bodies were equally immunostained. Interestingly, the vast majority of granular neurons in the dentate gyrus were stained with anti-phospho-*tau* antibodies: more than a half contained mini-Pick-like bodies. Yet pre-tangles and *tau*-immunoreactive inclusions, including mini-Pick-like bodies, were not stained with anti-α-tubulin and anti-β-tubulin antibodies. Mini-Pick-like bodies, and the vast majority of neurons, excepting rare neurofibrillary tangles, were not stained with anti-ubiquitin antibodies (data not shown).

3.2. Phospho-kinase immunoreactivity

Neuronal immunostaining was rarely observed with anti-MAPK/ERK-P antibodies. Yet, small punctate SAPK/JNK-P-immunoreactive cytoplasmic granules were present in about 1/3 of granular neurons in the dentate gyrus (Fig. 3 A, C). Immunoreactivity to p-38-P was observed in more than a half of dentate gyrus granular neurons (Fig. 3 A, D), and in many cortical and CA1 pyramidal neurons with pre-tangles (Fig. 3 B, E). No differences in GSK-3α/β immunostaining was found between neurons with and without abnormal *tau* deposits (data not shown). Mini-Pick-like bodies were decorated with anti-GSK-3β-P antibodies (Fig. 3 F). However, GSK-3β-P was rarely encountered in neurons with no mini-Pick-like bodies, including those of the frontal and temporal neocortex.

3.3. Biochemical studies

Biochemical studies of total homogenates and sarkosyl-insoluble fractions disclosed a pattern of two bands of phospho-*tau* of 68/66 kDa and 64 kDa in the

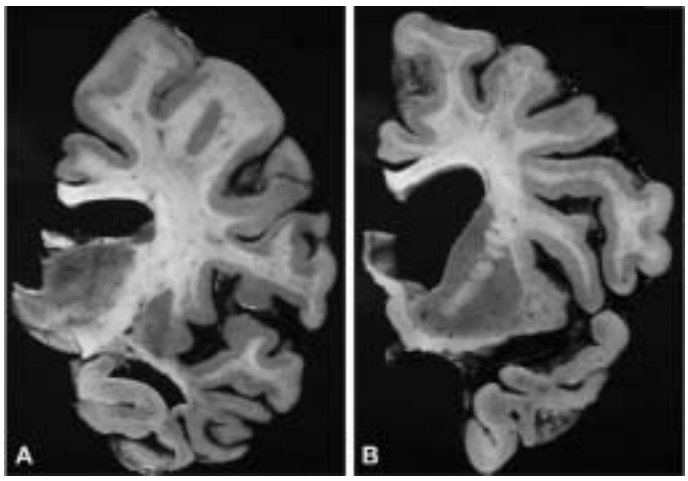


Fig. 1. Marked loss of neurons and spongiosis of the upper layers in the frontal cortex (A). This is accompanied by ballooned neurons containing ?B-crystallin (B). No apparent cell loss occurs in the dentate gyrus although a few granule cells contain pale small cytoplasmic inclusions (D, arrow). Paraffin sections; CC: cerebral cortex; DG: dentate gyrus. A, bar $= 25$ μm. B and C, bar in $C = 10$ μm.

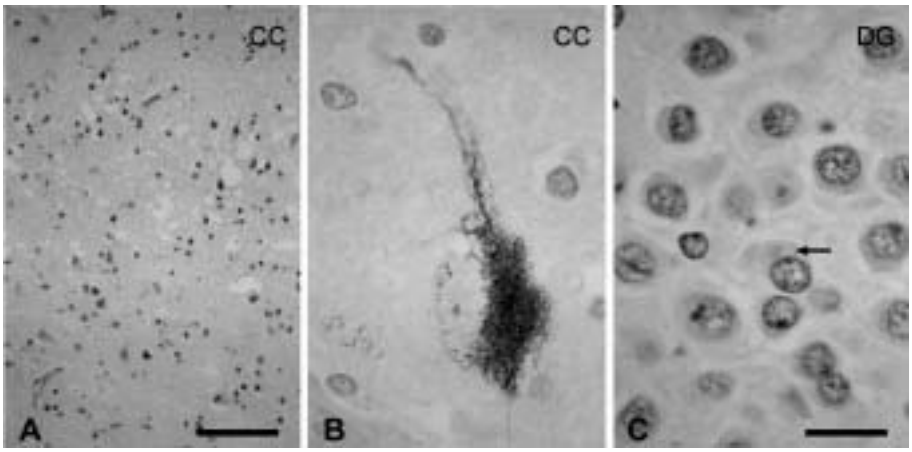


Fig. 2. Tau-immunoreactive neurons are seen in the frontal cortex (A), entorhinal cortex (B), CA1 area of the hippocampus (C), striatum (D), dentate gyrus (E) and Meynert nucleus (F). Most *tau*-immunoreactive neurons are pre-tangles showing perinuclear immunoreactive halos and cytoplasmic small inclusion roughly resembling mini-Pick bodies, which are particularly abundant in neurons of the dentate gyrus. In addition, *tau*-immunoreactive astrocytes are found in the cerebral cortex (G, H), and *tau*-immunoreactive oligodendrocytes in the white matter (I). Paraffin sections slightly counterstained with haematoxylin. CC: cerebral cortex; EC: entorhinal cortex; CA1: area of the hippocampus; str: striatum; DG: dentate gyrus; Mey: Meynert nucleus; As: astrocytes; WM: white matter. Bar $= 10$ μm.

hippocampus and white matter. The same results were obtained with the antibodies to phospho-tauThr181, phospho-tauSer262 and phospho-tauSer422 (Fig. 4).

Kinase expression in the hippocampus was examined in parallel in AD and P301L mutation for comparative purposes. Sarkosyl-insoluble fractions in AD, but not in P301L, were enriched in p38-P and GSK-3β-P. Moreover, p38-P-immunoreactive bands of high molecular weight were not found in the P301L mutation. Finally, the largest amount of GSK-3β-P was found in the sarkosyl-insoluble fraction in AD (Fig. 5).

4. Discussion

Tau accumulation in the patient with a P301L mutation in the *tau* gene and included in the H1 haplotype was found in neurons with pre-tangles, and astrocytes and oligodendrocytes through the brain. The most characteristic feature was *tau* immunoreactivity decorating the perinuclear region and small cytoplasmic aggregates designed as mini-Pick-like bodies, mainly in the dentate gyrus. Similar inclusions were reported in seminal cases [31] and have been recently examined by

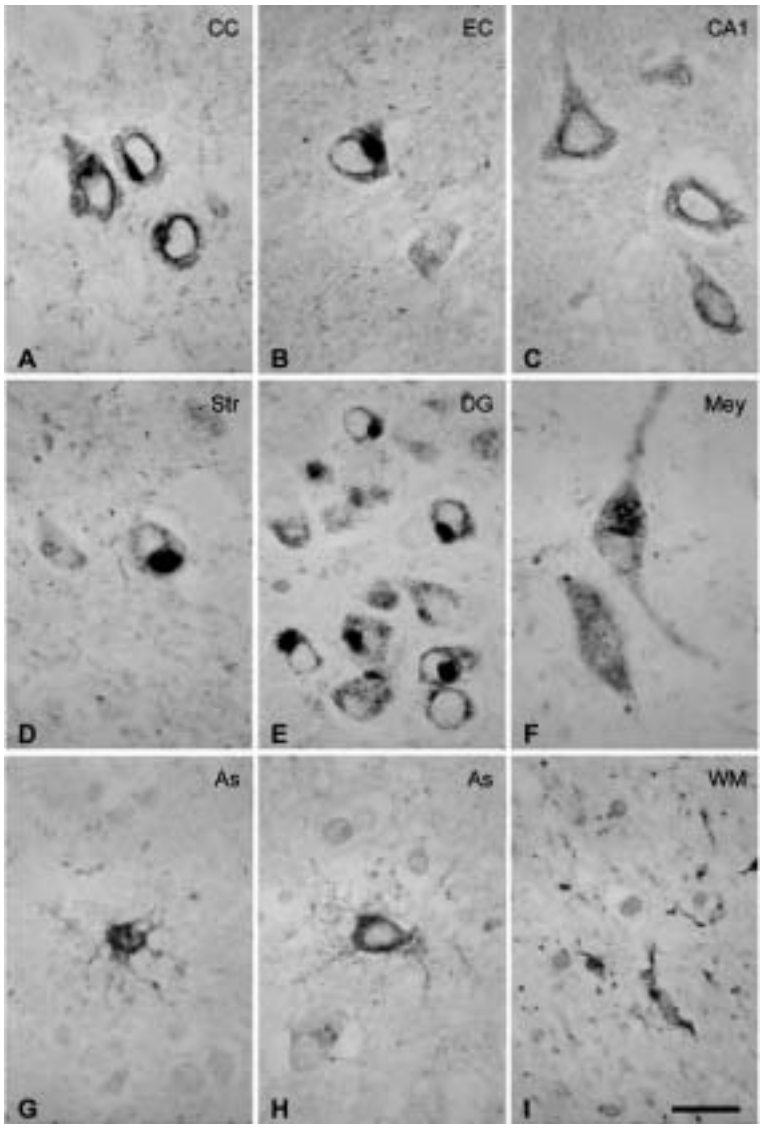


Fig. 3. Mini-Pick-like bodies in the dentate gyrus (A) and pre-tangle neurons in the hippocampus (B) are associated with small punctate SAPK/JNK-P (C) and p38-P (D and E) cytoplasm inclusions. A number of mini-Pick bodies are also stained with anti-GSK-3β-P antibodies (F). Paraffin sections, slightly counterstained with haematoxylin. Bar = 10 μm.

Adamec et al. [1]. Perinuclear *tau*-positive immunoreactivity also occurs in FTDP-17 with a P301S mutation in the *tau* gene [8]. Pick-body-like neuronal inclusions have also been found in association with a G389R mutation in the *tau* gene in the context of FTDP-17 [36].

The sites of *tau* phosphorylation, as seen by using a panel of phospho-specific anti-*tau* antibodies, are similar in P301L tauopathy and other sporadic and familial tauopathies [11,15]. Phosphorylation sites include Thr181, Ser199, Ser202, Ser214, Ser262, Ser396 and Ser422, and those recognized by the antibody AT8 (Ser202 andThr205). The present results have shown increased SAPK/JNK-P and p-38-P expression in association with *tau* deposits, whereas only a few neurons have been stained with the anti-MAPK/ERK-P antibodies. Phosphorylation of *tau* in the P301L mutation probably depends on the same kinases that phosphoylate *tau* in other tauopathies, including Alzheimer's disease, progressive supranuclear palsy, corticobasal degeneration, Pick's disease, argyrophilic grain disease and FTDP-17 [2,11–15,25,34,35,43–45]. Therefore, it is reasonable to conclude that mutated *tau* facilitates *tau* phosphorylation through a mechanism that is common to other tauopathies. In addition, GSK-3β-PSer9

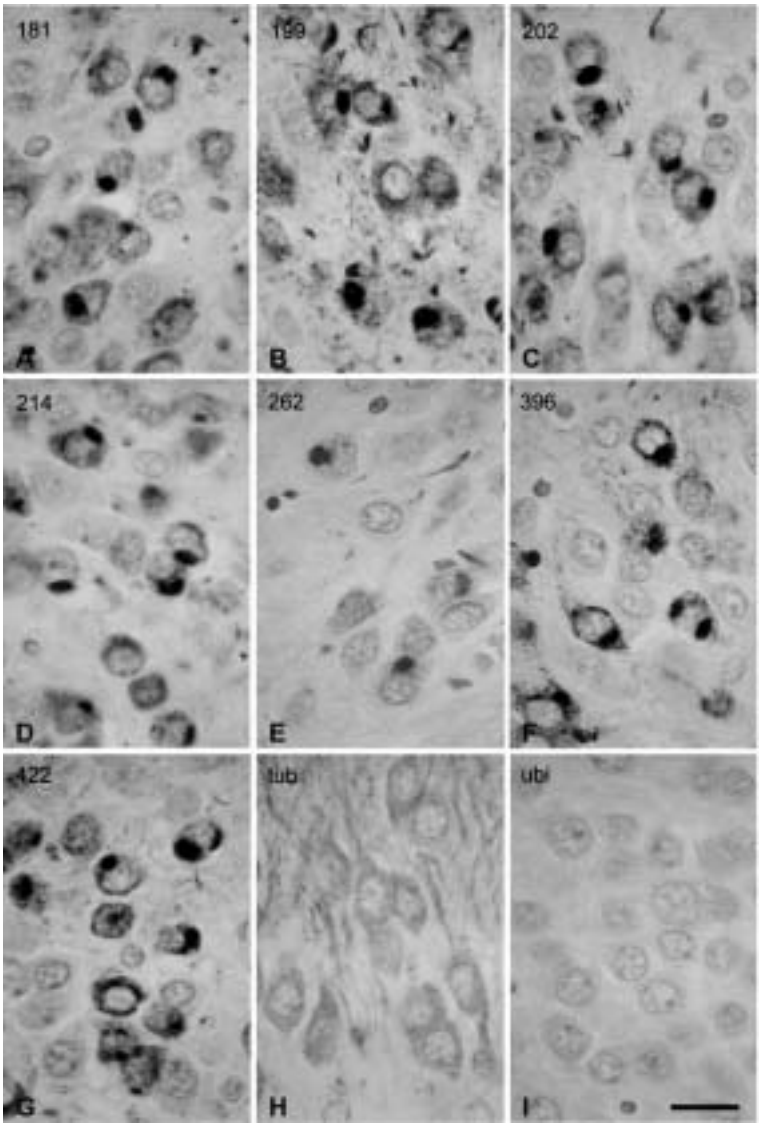


Fig. 4. Western blots of sarkosyl-insoluble fractions of the white matter and hippocampus showing a pattern of two bands of 68/66 kDa and 64 kDa by using phospho-specific anti-*tau* antibodies Thr181, Ser262 and Ser422.

is present in neurons and glial cells in sporadic and familial tauopathies [11,12,15,34]. It is interesting to note that GSK-3β-PSer9 decorates a few neurons in the cerebral cortex but the majority of neurons with mini-Pick-like bodies in the dentate gyrus in the P301L mutation. Phosphorylation of GSK-3β at Ser9 inactivates the kinase and then the capacity to phosphorylate substrates, but GSK-3β-PSer9 also prevents cell death by apoptosis in several paradigms [9]. It is feasible that multiple signals can be triggered by GSK-3 depending on the state and site of phosphorylation [5,24]. Granule cells in the dentate gyrus are largely preserved in number whereas neurons in the upper neocortical layers are devastated. It is tempting to speculate that neurons expressing GSK-3β-PSer9 are best equipped to cancel cell death programs in P301L tauopathy.

Although phosphorylation sites in *tau* and expression of kinases in target cells kinases are similar in the P301L mutation and other sporadic and familial tauopathies, it is important to note that *tau* deposits differ from those encountered in AD and PiD. A major point is the lack of staining with anti-ubiquitin antibodies in the vast majority of *tau*-containing neurons, excepting the few with neurofibrillary tangles, and the lack of ubiquitination of the perinuclear halo and mini-Pick-like bodies in P301L tauopathy, as previously stressed by Adamec

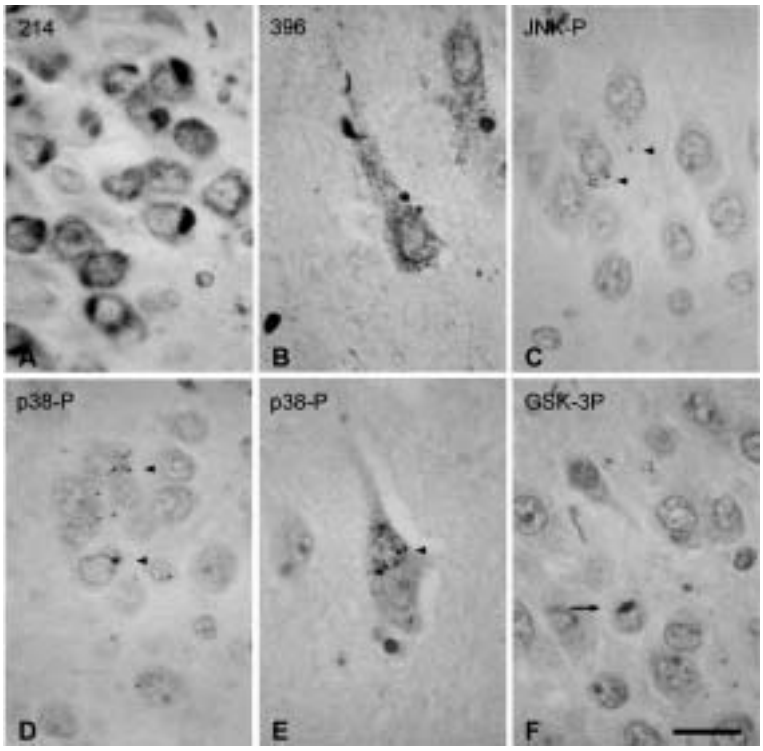


Fig. 5. Western blots of sarkosyl-insoluble (PHF) fractions and total homogenates of the hippocampus in Alzheimer's disease (AD) and P310L mutation. Results in AD are representative of four cases. Sarkosyl-insoluble fractions in AD are enriched in p38-P and GSK-3β-PSer9. Several bands of high molecular weight, reminiscent of those of phospho-*tau* in PHF fractions are seen in AD but not in the P310L mutation.

et al. [1]. Abnormal *tau* deposition in neurons is neither associated with abnormal accumulation of α-tubulin and β-tubulin, as occurs in neurofibrillary tangles in AD and Pick bodies in PiD. These findings further support the notion of structural differences between neuronal *tau* inclusions in AD and PiD, and P301L tauopathy. Pre-tangle neurons that are a characteristic feature in AGD are not ubiquitinated and do not recruit β-tubuline with this observation, p38-P and GSK-3β-P are enriched in sarkosyl-insoluble fractions in AD, but not in the P310L mutation. Moreover, p38-P bands of high molecular weight indicating either the formation of aggregates or abnormal cross-reaction with phospho-*tau* do occur in AD, but not in P310L mutation. Finally, higher GSK-3β-P expression is found in the sarkosyl-insoluble fraction in AD whereas the contrary occurs in P310L mutation. Interestingly, the pattern of kinases in subcellular fractions in P310L mutation resembles that found in argyrophilic grain disease [11] in which, in addition to grains, pre-tangles rather than tangles are the major pathological abnormality. Together these results point to differences in solubility of *tau* and associated kinases in P310L mutation when compared with AD.

Acknowledgements

This work was supported by grants FIS P1020004 and SAF-2001-4681E, and by the CIEN network project. We wish to thank T. Yohannan for editorial assistance.

References

[1] E. Adamec, J.R. Murrell, M. Takao, W. Hobbs, R.A. Nixon, B. Ghetti and J.P. Vonsattel, P301L tauopathy: confocal immunofluorescence study of perinuclear aggregation of the mutated protein, *J. Neurol. Sci.* **200** (2002), 85–93.

[2] C. Atzori, B. Ghetti, R. Piva, A.N. Srinivasan, P. Zolo, M.B. Delisle, S.S. Mirra and A. Migheli, Activation of JNK/p38 pathway occurs in diseases characterized by *tau* protein pathology and is related to *tau* phosphorylation but not to apoptosis, *J. Neuropathol. Exp. Neurol.* **60** (2001), 1190–11197.

[3] S. Barghorn, Q. Zheng-Fischofer, M. Ackmann, J. Biernat, M. von Bergn, E.M. Mandelkow and E. Mandelkow, Structure, microtubule interactions and paired helical filament aggregations by *tau* mutants of frontotemporal dementias, *Biochemistry* **39** (2000), 11714–11721.

[4] B.J. Bassam, G. Caetano-Anolles and P.M. Gresshoff, Fast and sensitive staining of DNA in polyacrylamide gels, *Annal. Biochem.* **196** (1991), 80–83.

[5] R.V. Bhat and S.L. Budd, GSK-3beta signaling: casting a wide net in Alzheimer's disease, *Neurosignals* **11** (2002), 251–261.
[6] T.D. Bird, D. Nochlin, P. Poorkaj, M. Charrier, J. Kayre, H. Payani, E. Reskind, T.H. Lampe, E. Nemens, P.S. Boyer and G.D. Schellenberg, A clinical pathological comparison of three families with frontotemporal dementia and identical mutations in the *tau* gene (P301L), *Brain* **122** (1999), 741–756.
[7] L. Buée, T. Bussi¤¤re, V. Buée-Cherrer, A. Delacourte and P.R. Hof, Tau protein isoforms, phosphorylation and role in neurodegenerative disorders, *Brain Res Rev* **33** (2000), 95–130.
[8] O. Bugiani, J.R. Murrell, G. Giacone, M. Hasegawa, G. Ghigo, M. Tabaton, M. Morbin, A. Primavera, F. Carella, C. Solaro, M. Grisoli, M. Savoiardo, M.G. Spillantini, F. Tagliavini, M. Goedert and B. Ghetti, Frontotemporal dementia and corticobasal degeneration in a family with a P301S mutation in *tau*, *J. Neuropathol. Exp. Neurol.* **58** (1999), 667–677.
[9] P. Cohen and S. Frame, The renaissance of GSK3, *Nat. Rev.* **2** (2001), 769–776.
[10] C. Conrad, C. Vianna and M. Freeman et al., A polymorphic gene nested within an intron of the *tau* gene: implications for Alzheimer's disease, *Proc Natl Acad Sci USA* **99** (2002), 7751–7756.
[11] I. Ferrer, M. Barrachina, M. Tolnay, M.J. Rey, N. Vidal, M. Carmona, R. Blanco and B. Puig, Phosphorylated protein kinases associated with neuronal and glial *tau* deposits in argyrophilic grain disease, *Brain Pathol* **13** (2003), 62–78.
[12] I. Ferrer, M. Barrachina and B. Puig, Glycogen synthase kinase-3 (GSK-3) is associated with neuronal and glial hyperphosphorylated *tau* deposits in Alzheimer's disease, Pick's disease, progressive supranuclear palsy and corticobasal degeneration, *Acta Neuropathol* **104** (2002), 658–664.
[13] I. Ferrer, R. Blanco, M. Carmona and B. Puig, Phosphorylated mitogen-activated protein kinase (MAPK/ERK-P), protein kinase of 38 kDa (p38-P), stress-activated protein kinase (SAPK/JNK-P), and calcium/calmodulin-dependent kinase II (CaM kinase II) are differentially expressed in *tau* deposits in neurons and glial cells in tauopathies, *J. Neural. Transm.* **108** (2001), 1397–1415.
[14] I. Ferrer, R. Blanco, M. Carmona, R. Ribera, E. Goutan, B. Puig, M.J. Rey, A. Cardozo, F. Viñals and T. Ribalta, Phosphorylated MAP kinase (ERK1, ERK2) expression is associated with early *tau* deposition in neurones and glial cells, but not with increased nuclear DNA vulnerability and cell death, in Alzheimer disease, Pick's disease, progressive supranuclear palsy and corticobasal degeneration, *Brain Pathol* **11** (2001), 144–158.
[15] I. Ferrer, P. Pastor, M.J. Rey, E. Muñoz, B. Puig, E. Pastor, R. Oliva and E. Tolosa, Tau phosphorylation and kinase activation in familial tauopathy linked to delN296 mutation, *Neuropathol. Appl. Neurobiol.* **29** (2003), 23–34.
[16] B. Ghetti, M.L. Hutton and Z.K. Wszolek, Frontotemporal dementia and parkinsonism linked to chromosome 17 associated with *tau* gene mutations (FTDP-17T), in: *Neurodegeneration: The molecular pathology of dementia and movement disorders*, D. Dickson, ed., ISN Neuropath Press, Basel 2003, pp. 86–102.
[17] M. Goedert, R. Jakes and E. Vanmechelen, Monoclonal antibody AT8 recognizes *tau* protein phosphorylated at both serine 202 and threonine 205, *Neurosci. Lett.* **189** (1995), 167–169.
[18] J. Götz, Tau and transgenic animal models, *Brain Res. Rev.* **35** (2001), 266–286.
[19] J. Götz, F. Chen, R. Barmettler and R.M. Nitsch, Tau filament formation in transgenic mice expressing P301L *tau*, *J. Biol. Chem.* **276** (2001), 529–534.
[20] M. Hasegawa, M.J. Smith and M. Goedert, Tau proteins with FTDP-17 mutations have a reduced ability to promote microtubule assembly, *FEBS Lett* **437** (1998), 207–210.
[21] M. Hutton, C.L. Lendon, P. Rizzu, M. Baker, S. Froelich, H. Houlden, S. Pickering-Brown, S. Chakraertz, A. Isaacs, A. Gover, J. Hackett, J. Adamson, S. Lincoln, D. Dickson, P. Davies, R.C. Petersen, M. Stevans, I. de Graaf, E. Wantens, J. van Baren, M. Hillebrand, J.M. Joosse, V. Nowotny and P. Heutriak et al., Association of a missense and 5' splice-site mutations in *tau* with the inherited dementia FTDP-17, *Nature* **393** (1998), 702–705.
[22] M. Hutton, H. Lewis, D. Dickson, S.H. Yen and E. McGowen, Analysis of tauopathies with transgenic mice, *Trends Mol. Med.* **7** (2001), 467–470.
[23] E.M. Ingram and M.G. Spillantini, Tau gene mutations: dissecting the pathogenesis of FTDP-17, *Trends Mol. Med.* **8** (2002), 556–562.
[24] M.D. Kaytor and H.T. Orr, The GSK3β signaling cascade and neurodegenerative disease, *Curr. Opin. Neurobiol.* **12** (2002), 275–278.
[25] R.B. Knowles, J. Chin, C.T. Ruff and B.T. Hyman, Demonstration by fluorescence resonance energy transfer of a close association between activated MAP kinase and neurofibrillary tangles: Implications for MAP kinase activation in Alzheimer disease, *J. Neuropathol. Exp. Neurol.* **58** (1999), 1090–1098.
[26] T. Kobayashi, H. Mori, Y. Okuma, D.W. Dickson, N. Cookson, Y. Tsuboi, Y. Motoi, R. Tanaka, N. Miyashita, M. Anno, H. Narabayashi and Y. Mizzuno, Contrasting genotypes of the *tau* gene in two phenotypically distinct patients with P301L mutation of frontotemporal dementia and parkinsonism linked to chromosome 17, *J. Neurol.* **249** (2002), 669–675.
[27] M.V.Y. Lee, M. Goedert and J.Q. Trojanowski, Neurodegenerative tauopathies, *Annu. Rev. Neurosci.* **24** (2001), 1121–1159.
[28] J. Lewis and D.V. Dickson, Transgenic animal models of tauopathies, in: *Neurodegeneration: The molecular pathology of dementia and movement disorders*, D. Dickson, ed.,ISN Neuropath Press, Basel 2003, pp. 150–154.
[29] J. Lewis, E. McGowan, J. Rockwood, H. Melrose, P. Nacharaju, M. van Slegtenhorst, K. Gwinn-Hardy, M. Paul Murphy, M. Baker, X. Yu, J. Hardy, A. Corral, W.L. Lin, S.H. Yen, D.W. Dickson, P. Davies and M. Hutton, Neurofibrillary tangles, amyotrophy and progressive motor disturbance in mice expressing mutant (P301L) *tau* proteins, *Nat. Genet.* **25** (2000), 402–405.
[30] W.L. Lin, J. Lewis, S.H. Yen, M. Hutton and D.W. Dickson, Filamentous *tau* oligodendrocytes and astrocytes of transgenic mice expressing the human *tau* isoform with the P301L mutation, *Am. J. Pathol.* **162** (2003), 213–218.
[31] S.S. Mirra, J.R. Murrell, M. Gearing, M.G. Spillantini, M. Goedert, A. Crowther, A.I. Levey, R. Jones, J. Green, J.M. Shioffner, B.H. Wainer, M.L. Schmidt, J.Q. Trojanowski and B. Guetti, Tau pathology in a family with a P301L mutation in *tau*, *J. Neuropathol. Exp. Neurol.* **58** (1999), 335–345.
[32] J.R. Murrell, M.G. Spillantini, P. Zolo, M. Guazzelli, M.J. Smith, M. Hasegawa, F. Redi, A. Crowther, P. Pietrini, B. Ghetti and M. Goedert, Tau gene mutation G389R causes a tauopathy with abundant Pick Body-like inclusions and axonal deposits, *J. Neuropathol. Exp. Neurol.* **58** (1999), 1207–1226.
[33] Z.S. Nasreddine, M. Loginov, L.N. Clark, J. Lamarche, B.L. Miller, A. Lamontagne, V. Zhurakeva, M.V. Lee, K.L. Wilhelmen and P.H. Geshwind, From genotype to phenotype: a

clinical pathological, and biochemical investigation of frontotemporal dementia and parkinsonism (FTDP-17) caused by the P301L mutation, *Ann. Neurol.* **45** (1999), 704–715.

[34] J.J. Pei, E. Braak, H. Braak, I. Grundke-Iqbal, K. Iqbal, B. Winblad and R.F. Cowburn, Distribution of glycogen synthase kinase 3 beta (GSK-3β) for Alzheimer disease neurofibrillary tangle, *J. Neuropathol. Exp. Neurol.* **58** (1999), 1010–1119.

[35] G. Perry, H. Roder, A. Nunomura, A. Takeda, A.L. Friedlich, X. Zhu, A.L. Raina, N. Holbrook, S.L. Siedlak, P.L.R. Harris and M.A. Smith, Activation of neuronal extracellular receptor kinase (ERK) in Alzheimer disease links oxidative stress to abnormal *tau* phosphorylation, *NeuroReport* **10** (1999), 2411–2415.

[36] P. Rizzu, M. Joosse, R. Ravid, A. Hoogeveen, W. Kamphorst, J.C. van Swieten, R. Willemsen and P. Huetink, Mutation-dependent aggregation of *tau* protein and its selective depletion from the soluble fraction in brain of p310L FTDP-17 patients, *Hum. Mol. Genet.* **9** (2000), 3075–3082.

[37] M.G. Spillantini, R.A. Crowther, W. Kamphorst, P. Heutink and J.C. van Swieten, Tau pathology in two Dutch families with mutations in the microtubule-binding region of *tau*, *Am. J. Pathol.* **153** (1998), 1359–1363.

[38] M.G. Spillantini and M. Goedert, Tau protein pathology in neurodegenerative diseases, *Trends Neurosci* **21** (1998), 428–433.

[39] T. Togo, N. Sahara, S.H. Yen, N. Cookson, T. Ishizawa, M. Hutton, R. De Silva, A. Lees and D.W. Dickson, Argyrophilic grain disease is a sporadic 4-repeat tauopathy, *J. Neuropathol. Exp. Neurol.* **61** (2002), 547–556.

[40] M. Tolnay and A. Probst, Review: Tau protein: pathology in Alzheimer's disease and related disorders, *Neuropathol. Appl. Neurobiol.* **25** (1999), 171–187.

[41] R.H. Walker, J. Friedman, J. Wiener, R. Hobler, K. Gwinn-Hardy, A. Adam, J. DeWolfe, R. Gibbs, M. Baker, M. Farrer, M. Hutton and J.A. Hardy, A family with a *tau* P301L mutation presenting with parkinsonism, *Parkinsonism Relat Disord* **9** (2002), 121–123.

[42] S. H. Yen, M. Hutton, M. deTure, L.W. Ko and P. Nacharaju, Fibrillogenesis of *tau*: insights from *tau* missense mutations in FTDP-17, *Brain Pathol* **9** (1999), 695–705.

[43] X. Zhu, H.G. Lee, A.K. Raina, G. Perry and M.A. Smith, The role of mitogen-activated protein kinase pathways in Alzheimer's disease, *Neurosignals* **1** (2002), 270–281.

[44] X. Zhu, A.K. Raina, C.A. Rottkamp, G. Aliev, G. Perry, H. Boux and M.A. Smith, Activation and re-distribution of c-Jun N-terminal kinase/stress activated protein kinase in degenerating neurons in Alzheimer's disease, *J. Neurochem.* **76** (2001), 435–441.

[45] X. Zhu, C.A. Rottkamp, H. Boux, A. Takeda, G. Perry and M.A. Smith, Activation of p38 kinase links *tau* phosphorylation, oxidative stress, and cell cycle-related events in Alzheimer disease, *J. Neuropathol. Exp. Neurol.* **59** (2000), 880–888.

Acta Neuropathol (2003) 106 : 419–435
DOI 10.1007/s00401-003-0756-4

REGULAR PAPER

I. Ferrer · I. Hernández · M. Boada · A. Llorente
M. J. Rey · A. Cardozo · M. Ezquerra · B. Puig

Primary progressive aphasia as the initial manifestation of corticobasal degeneration and unusual tauopathies

Received: 7 April 2003 / Revised: 24 June 2003 / Accepted: 24 June 2003 / Published online: 29 August 2003

Abstract The clinical, neuroradiological, neuropathological and biochemical findings in four patients with primary progressive aphasia and tauopathy are described. The aphasic syndrome preceded by several years the appearance of other symptoms in every case. Asymmetrical apraxia with alien hand phenomenon occurred in one case. Frontotemporal symptoms occurred in three cases, but progressed to dramatic cognitive devastation in only one of these. Generalized dementia consistent with probable Alzheimer's disease (AD) developed with time in another. Cerebral computer tomography scans, magnetic resonance imaging and SPECT studies revealed marked asymmetries in one case, and showed nonspecific cerebral atrophy in the remaining ones. The neuropathological examination revealed typical corticobasal degeneration (CBD) in one case; CBD and AD in another; and atypical CBD, argyrophilic grain disease (AGD) and α-synucleinopathy consistent with Parkinson's disease in a third. Unique neuropathological findings were found in the remaining case. This was characterized by severe cerebral atrophy, marked neuronal loss in the cerebral cortex and abnormal tau deposition in neurons of the cerebral cortex, diencephalon and brain stem. Ballooned neurons, Pick bodies, generalized cortical neurofibrillary tangles and astrocytic plaques were absent. However, massive globular inclusions, containing phospho-tau, occurred in glial cells, mainly oligodendrocytes, in the white matter. Biochemical studies of frontal homogenates revealed four bands of 73/74, 68, 64 and 60 kDa of phosphorylated tau (using antibodies recognizing phospho-tau Thr181, Ser262 and Ser422) in the patient with AD and CBD, suggesting a predominant AD pattern in this case. Two bands of 68 and 64 kDa of phospho-tau were recovered in the sarkosyl-insoluble fraction in the other three cases. This pattern is similar to that found in CBD, progressive supranuclear palsy and AGD. Taken together, the present series further supports pure and combined CBD as causes of primary progressive aphasia, and they extend the hypothesis that primary progressive aphasia may be the initial symptom of distinct tauopathies.

Keywords Primary progressive aphasia · Corticobasal degeneration · Tauopathy · Argyrophilic grain disease · α-Synuclein

I. Ferrer (✉) · B. Puig
Institut de Neuropatologia, Servei Anatomia Patològica,
Hospital Princeps d'Espanya (Bellvitge CSUB),
carrer Feixa Llarga sn, 08907 Hospitalet de Llobregat, Spain
Tel.: +34-93-4035808
e-mail: 8082ifa@comb.es

I. Ferrer · M. J. Rey · A. Cardozo · M. Ezquerra
Banc de Teixits Neurològics,
Universitat de Barcelona/Hospital Clinic, Barcelona, Spain

I. Hernández · M. Boada · A. Llorente
Fundaciò ACE, Institut Català de Neurociències Aplicades,
Barcelona, Spain

I. Hernández
SAP Nou Barris, ICS, CAPII Chafarinas Neurología,
Barcelona, Spain

M. Boada
Servei de Neurologia,
Hospital General Universitari Valle Hebrón, Barcelona, Spain

Introduction

The term 'primary progressive aphasia' was introduced to describe a syndrome characterized by progressive worsening of language with preservation of the activities of daily life and non-verbal abilities [52, 53]. Although the seminal reports emphasized lack of dementia, subsequent observations have shown variable cognitive impairment and dementia in the majority of cases of primary progressive aphasia.

Several neurological diseases may produce primary progressive aphasia. These include Alzheimer's disease (AD) [3, 28, 37, 60], Pick's disease (PiD) [27, 29, 81], and Creutzfeldt-Jakob disease [51, 66, 82]. Nonspecific spongiform changes and astrocytic gliosis of the upper cortical layers have also been reported [39, 40, 42, 43, 48, 58, 65, 67, 77]. Cortical achromatic neurons have been reported in some cases. Spongy change in the frontal cortex and

striatum accompanied by abnormally ubiquitinated neurites in the cerebral cortex [41] is a rare condition associated with primary progressive aphasia. Corticobasal degeneration (CBD) [24], formerly named corticodentatonigral degeneration with neuronal achromasia [61], is a common cause of primary progressive aphasia [1, 32, 45, 55, 62, 83]. Finally, progressive supranuclear palsy (PSP) may develop with progressive nonfluent aphasia [7, 56].

Together, the cumulative clinical and pathological evidence supports the concept that primary progressive aphasia is not uncommon in a variety of diseases with frontotemporal atrophy, including AD, PiD, frontotemporal dementia (FTD), PSP and CBD [40]. The majority of these diseases are associated with impaired tau metabolism, usually manifested as an accumulation of abnormally phosphorylated tau in neurons and glial cells. However, a subgroup of FTD cases have decreased tau levels in total brain homogenates [79]. Abnormal tau phosphorylation and deposition in the cytoplasm of neurons and glial cells are major biochemical and structural anomalies in AD, CBD, PiD and PSP [11, 12, 21, 26, 47]. However, AD, CBD, PiD and PSP have clinical, neuropathological and biochemical peculiarities. Phosphorylation of tau occurs at different sites, and phosphorylated bands from homogenates of fractions enriched with paired helical filaments or with abnormal filaments show particular patterns. AD displays four bands of 73/74, 68, 64 and 60 kDa, whereas CBD and PSP are resolved into two bands of 68 and 64 kDa. PiD shows two bands of 64 and 60 kDa [11, 12, 20, 26, 47]. Inherited tauopathies associated with mutations or deletions in the *tau* gene are manifested as PiD-, PSP- or FTD-like syndromes, and can be resolved into different band patterns on Western blots of brain homogenate fractions enriched in abnormal filaments [12, 33, 47]. Finally, argyrophilic grain disease (AGD) is another tauopathy with invariable limbic involvement that produces two bands of 68 and 64 kDa [22, 72, 75].

Detailed clinical and neuropathological studies in patients with primary progressive aphasia and sporadic tauopathy are important because they help to increase our understanding of the spectrum of diseases with abnormal tau accumulation that manifest as primary progressive aphasia. The present study is focused on four patients with long-lasting primary progressive aphasia followed by cognitive impairment in which the neuropathological and biochemical study disclosed singular tauopathies.

Case reports

Case 1

The patient was a 66-year-old right-handed woman with a primary school education. There was no familiy history of neurological disease. Moderate hypertension was controlled with diet and medication. In 1987, she complained of a transient episode of difficulty in speaking. A brain CT scan showed mild cerebral atrophy without other anomalies. She was diagnosed as having suffered from a transitory ischemic attack and received anti-aggregant drugs.

In 1990, the patient noticed slowly progressive difficulties in expressing herself. A brain CT scan revealed a slight increase of the sylvian fissures compared with the previous study carried out 3 years earlier. The cerebral magnetic resonance imaging (MRI) demonstrated cortical atrophy of the right perisylvian region and of the left temporal lobe, together with hyperintense periventricular signals in the white matter.

A neuropsychological study 2 years later revealed language worsening, but reading and writing comprehensions were preserved. A new study in April 1994 showed a reduction in fluency, with non-grammatical language and bucal and facial apraxia. Reading and writing remained intact. The MMSE showed scores of 18/30, and the patient was able to maintain daily activities including the full organization of the family life. The patient was diagnosed as suffering from progressive aphasia with slight damage of right frontal functions and buco-lingual apraxia. A cerebral SPECT performed in 1994 revealed inter-hemispheric asymmetry with decreased flow in the right frontal cortex.

By the end of 1995, spasmodic laughter and difficulties in swallowing had appeared. A new neuropsychological examination showed a deterioration of the visual-spatial and visual-constructive functions, and almost complete loss of the language; however, the patient was still capable of communicating by writing and by gestures (in order to say 'yes' she raised the thumb of her right hand, and placed it downwards to say 'no'). In addition, the neurological examination showed buco-facial-lingual apraxia, impaired swallowing of liquids and semi-liquids, and slight alteration of the voluntary ocular movements. The rest of the neurological examination was normal, showing no alterations in the pyramidal or extrapyramidal area. The cerebral SPECT revealed perisylvian attenuation, which was more marked on the right side.

In September 1996, the patient had trouble performing common domestic tasks, showed walking disorders, and suffered occasional urinary incontinence. Swallowing had worsened. The neuropsychological examination disclosed difficulties in understanding, as well as agraphia. The neurological examination showed bucal, facial and lingual apraxia, mild paresia of the motor ocular nerves, spasmodic laughter and sobbing, bilateral hyper-reflexia, grasping predominantly in the left side, axial rigidity, and left postural rigidity with walking apraxia. The patient did not suffer from behavioral disturbances excepting apathy, and her memory was preserved.

Dystonic movements appeared 1 year later. The neuropsychological examination in November 1997 evidenced phonemic disintegration with mutism, as well as major difficulty in swallowing and salivation, and reduced verbal comprehension. A cerebral SPECT showed, in addition to perisylvian attenuation, reduced signal in the posterior regions of the frontal and temporal lobes, which was more marked in the right hemisphere.

One year later, the patient was suffering from progressive worsening of mobility and cognitive deterioration. Her relatives asked for percutaneous feeding, which was started by the middle of 1998. A few months later, the patient was confined to bed, decorticated with generalized hyperreflexia and hypertonia but still able to recognize faces. During the following 2 years, the patient deteriorated and her mental status was very poor. The patient died in April 2002 at the age of 81, 12 years after the beginning of aphasia.

Case 2

The patient was a right-handed woman with limited education. There was no familiy history of neurological disease. She was 72 years old at the time of her first consultation. In May 1995 she presented for a progressive language disorder that had started 2 years earlier. The neuropsychological examination showed language alterations and difficulties in visual-spatial functions. The MMSE was 27/30. A cerebral CT scan showed slight cortical and subcortical cerebral atrophy. A cerebral SPECT disclosed dilatation of the ventricular system without regional cortical perfusion defects.

Language deterioration was accompanied by increased dependence on relatives to communicate aspects of current life. A new neuropsychological evaluation was performed in 1997, which showed a marked language alteration with loss of fluency and anomy. Linguistic understanding was better preserved, but deficits in the comprehension of complex orders were already detected at this time. The patient's writing was agraphic, and she had difficul-

ties in the written repetition of long sentences. Abstract reasoning skills were diminished and slight visual-spatial deficits were present.

The neuropsychological evaluation in November 1997 disclosed further deterioration of spontaneous language. Verbal understanding was preserved and reading comprehension was intact.

In August 1998, during a control examination, a mammary tumor was discovered, and a radical mastectomy was conducted, followed by chemotherapy and radiation therapy.

In February 2001, the patient required assisted feeding, because of severe buco-lingual apraxia. Marked rigidity predominated in the right side. In addition, cognitive impairment and loss of memory characterized the last months of her life. The patient died in April 2002 at the age of 78, 9 years after the beginning of aphasia.

Case 3

The patient was a right-handed man with no family history of neurological disease, who had suffered from progressive disorder of speech starting in 1994. This was followed 3 years later by a behavior disorder consisting of disinhibition and compulsive laughing, asymmetrical rigidity and apraxia of the upper extremity, anticipating alien hand phenomenon 2 years later. The CT scan performed in November 1999 disclosed moderate frontal, parietal and temporal atrophy, and enlargement of the left perisylvian region. The neurological status worsened during the year 2000 with marked deterioration of mood and personality. Social difficulties due to non-structured behavior contrasted with the preservation of memory and orientation, as well as with the recognition of persons and places. Language was extremely impoverished leading to practical mutism. Parkinsonism signs were not recorded at any time during the course of the disease. The patient died in November 2001 at the age of 73.

Case 4

The patient was a man aged 68 who developed, during the winter of 1990, progressive loss of language and verbal fluency contrasting with preserved comprehension, reading and naming skills. There was no familiy history of neurological disease. The neurological examination in 1993 revealed no additional abnormalities, except slight left rigidity and dystonia. A cerebral CT scan revealed moderate frontal, parietal and temporal atrophy. His mental status rapidly deteriorated during the following year, with loss of the capacity for learning new information, followed by loss of short-term memory, and decline of motor skills. The patient was mute, rigid, disconnected and confined to a wheel-chair by 1996. The patient survived until November 1997, dying at the age of 73.

Neuropathological methods

The post-mortem delay between death and tissue processing varied from 2 to 15 h. Frozen samples for biochemical studies were available in every case.

A complete neuropathological examination was carried out in every case on tissue that had been formalin-fixed for no less than 3 weeks; the tissue was then embedded in paraffin. De-waxed sections, 7 µm thick, were stained with hematoxylin and eosin and Luxol fast blue-Klüver Barrera, or processed for immunohistochemistry following the avidin-biotin-peroxidase method (ABC kit, Vectastain, Vector). After blocking endogenous peroxidase, the sections were incubated with normal serum and incubated at 4°C overnight with one of the primary antibodies. Antibodies to phosphorylated 170- or 200-kDa neurofilaments (clones BF10 and RT97, Boehringer-Mannheim) were used at dilutions of 1:100 and 1:50, respectively. Rabbit polyclonal antibodies to αB-crystallin (Novocastra) were used at a dilution of 1:500. Rabbit polyclonal antibodies to α-synuclein (Chemicon) were used at a dilution of 1:500. Antibodies to pan-tau (Sigma) were used at a dilution of 1:10. Antibodies to glial fibrillary acidic protein (Dako, Dakopatts), βA4-amyloid (Boehringer-Mannheim), and ubiquitin (Dako) were used at dilutions of 1:250, 1:5, and 1:200, respectively. In addition, immunohistochemistry to anti-phospho-specific tau antibodies was carried out on de-waxed sections first boiled in citrate buffer and then stored overnight at room temperature. The following anti-phospho-specific tau rabbit polyclonal antibodies were used: Thr181, Ser202, Ser214, Ser262, Ser396 and Ser422 (all of them from Calbiochem). The antibodies were used at a dilution of 1:100, excepting anti-phospho-tauThr181, which was used at a dilution of 1:250. Finally, the phosphorylation-independent tau antibody 7.51, which is raised against the binding region of tau [54], was used at a dilution of 1:500.

Gel electrophoresis and Western blotting

For gel electrophoresis and Western blotting, fresh samples of the frontal cortex were immediately obtained at autopsy, frozen in liquid nitrogen, and stored at –80°C until use.

A protocol to purify paired helical filaments in AD, described by Goedert et al. [26], was used in every case. Frozen samples of about 5 g were cut into pieces. They were gently homogenized in a glass tissue grinder in 10 vol (w/v) of cold suspension buffer consisting of 10 mM TRIS-HCl pH 7.4, 0.8 M NaCl, 1 mM EGTA, 10% sucrose, 0.1 mM phenylmethylsulfonyl fluoride, 2 µg/ml aprotinin,

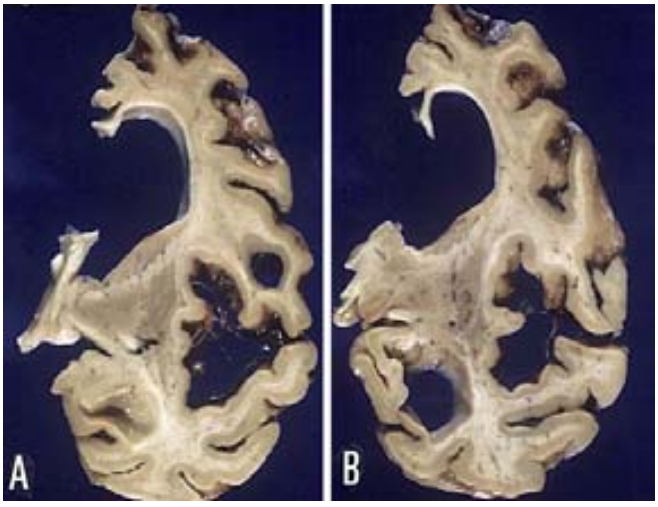


Fig. 1 Case 1. **A**, **B** Coronal sections of the brain showing marked atrophy of the frontal and temporal lobes, including the hippocampus and perirhinal cortex, and the perisylvian region. Marked enlargement of the lateral ventricles, reduced white matter and atrophy of the corpus callosum, and moderate atrophy of the caudate, putamen and thalamus, are additional remarkable features

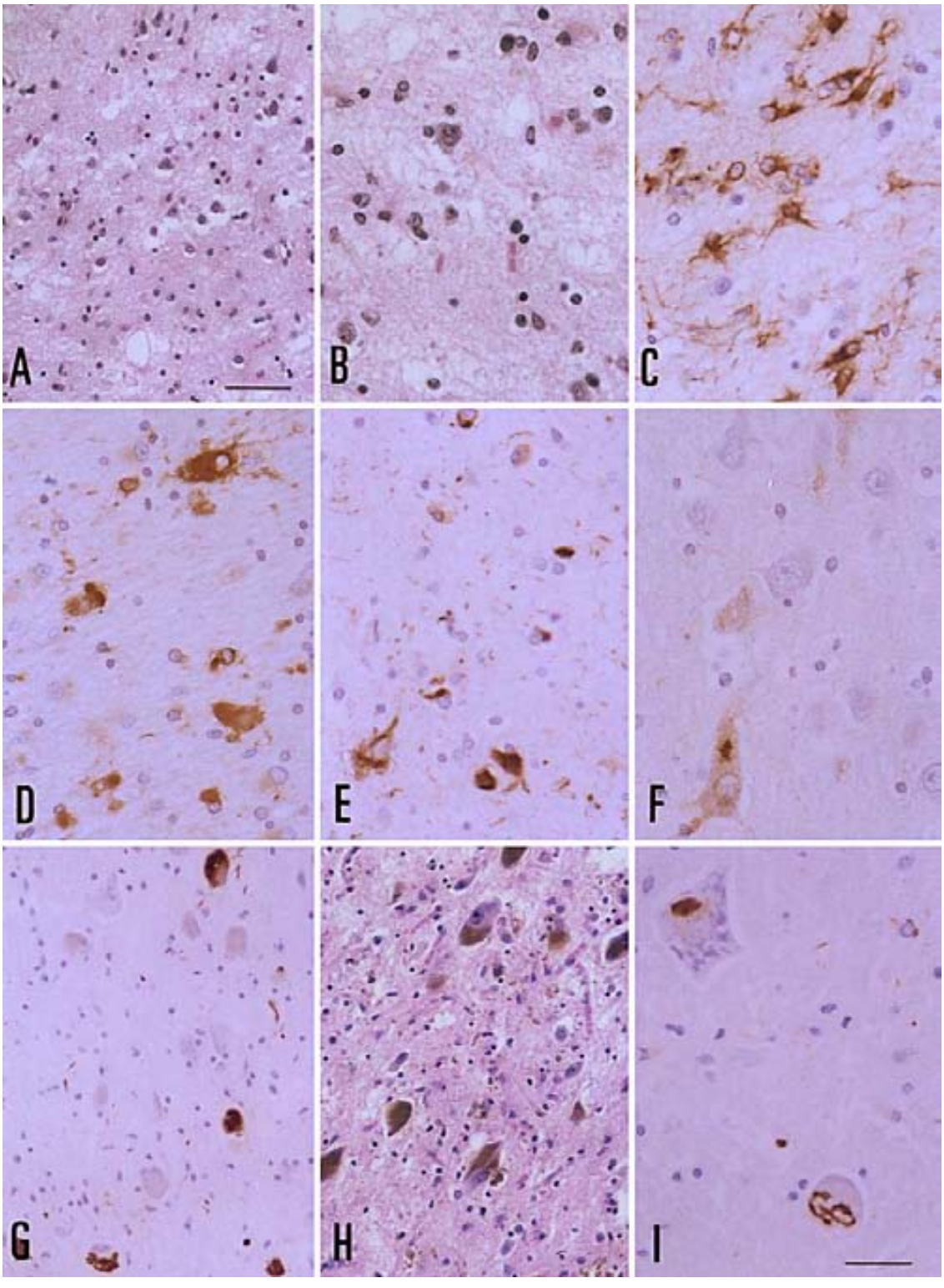


Fig. 2 Case 1. Marked neuron loss and spongiosis are found in the frontal cortex (**A**, **B**). This is accompanied by intense astrocytic gliosis, as revealed with glial fibrillary acidic protein immunohistochemistry (**C**), which is strongly immunostained with anti-αB-crystallin antibodies (**D**). Tau-immunoreactive deposits are found in scattered neurons in the cerebral cortex (**E**), hippocampus (**F**) and thalamus (**G**). Neuron loss and astrocytic gliosis is seen in the substantia nigra (**H**). Tau-immunoreactive inclusions are found in the dorsal nucleus of the vagus nerve (**I**). **A**, **B**, **H** Hematoxylin and eosin, **C**–**G**, **I** immunohistochemical sections slightly counterstained with hematoxylin; *bars* **A** 50 µm, **I** (also for **B**–**H**) 25 µm

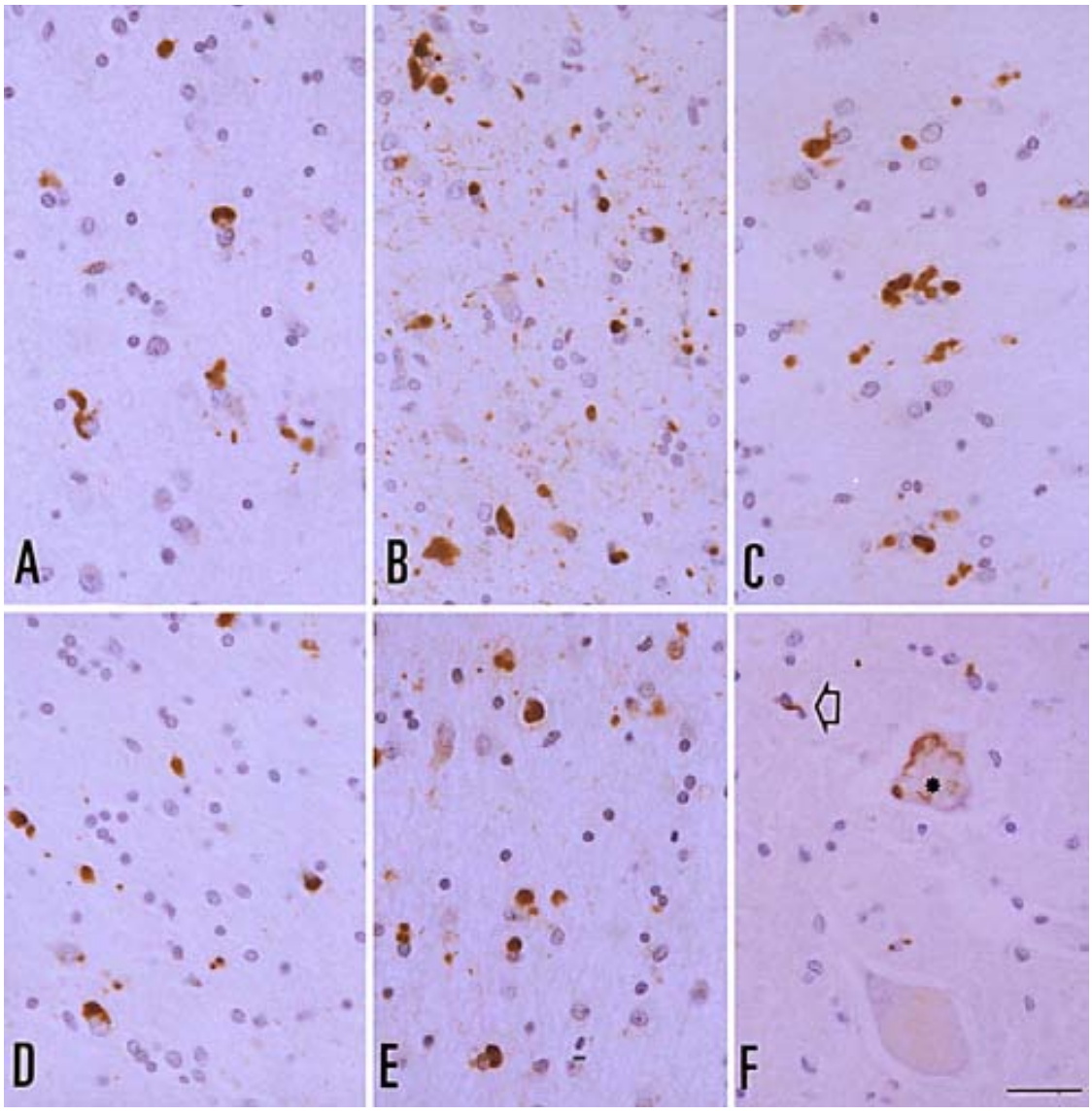


Fig. 3 Case 1. Tau-immunoreactive inclusions in glial cells in the white matter of the frontal lobe (**A**), parietal lobe (**B**), internal capsule (**C**) and corpus callosum (**D**). Glial inclusions are ubiquitinated (**E**). The majority of these inclusions are globular, but rare coiled bodies (*white arrow*) are seen in the vicinity of a neuron (*asterisk*) with a bizarre tau-immunoreactive inclusion in the anterior horn of the spinal cord (**F**). Immunohistochemical sections slightly counterstained with hematoxylin. *Bar* 25 µm

10 µg/ml leupeptin and 5 µg/ml pepstatin. The homogenates were first centrifuged at 20,000 *g*, and the supernatant (S1) was retained. The pellet (P1) was re-homogenized in 5 vol of homogenization buffer and re-centrifuged. The two supernatants (S1+S2) were then mixed and incubated with N-lauroylsarcosinate 1% for 1 h at room temperature while being shaken. Samples were then centrifuged for 1 h at 100,000 *g* in a Ti 70 Beckman rotor. Sarkosyl-insoluble pellets (P3) were re-suspended (0.2 ml/g starting material) in 50 mM TRIS-HCl pH 7.4. Protein concentrations were determined by the BCA method and 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was run using a maxi-protean system (Bio-Rad). 100–200 µg protein was loaded in each lane with loading buffer containing 0.125 M TRIS pH 6.8, 20% glycerol, 10% mercaptoethanol, 4% SDS and 0.002% bromophenol blue. Samples were heated at 95°C for 5 min prior to gel loading. Total frontal homogenates and fractions enriched with abnormal filaments were run in parallel. The proteins were then transferred to nitrocellulose membranes (Amersham) using an electrophoretic chamber system (Trans-Blot Electrophoretic Transfer Cell, Bio-Rad). Non-specific binding sites were blocked with TRIS-buffered saline solution pH 7.4 with 0.1% Tween-20 (TBST) containing 5% skimmed milk for 30 min, and incubated with one of the primary antibodies for 1 h at room temperature. Control of protein content in each lane was carried out by the staining of selected gels with Coomassie blue and of the membranes with Ponceau (Sigma).

The rabbit polyclonal antibody to phospho-tauThr181 was diluted 1:250. The rabbit polyclonal antibodies anti-

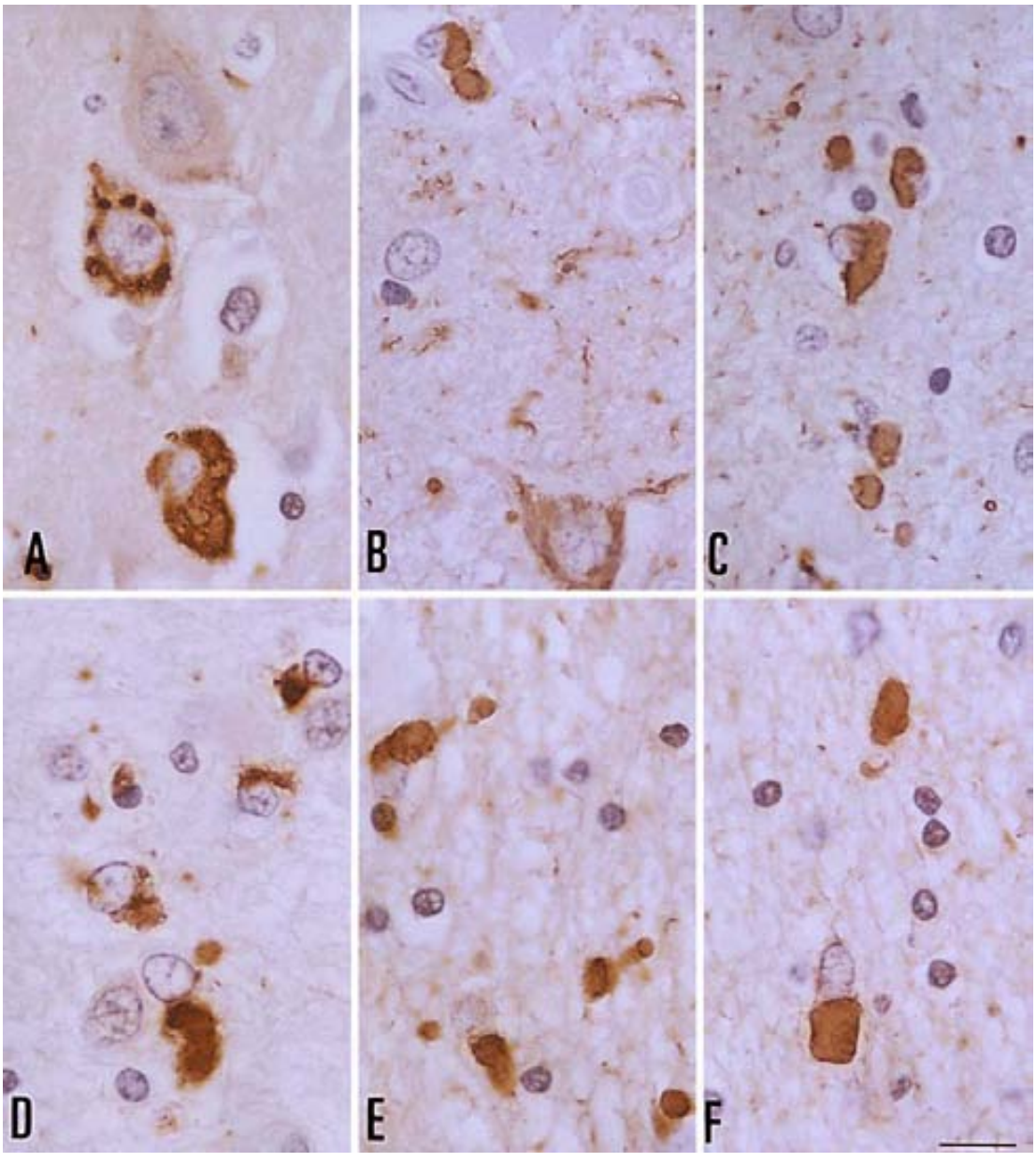


Fig. 4 Case 1. Tau-immunoreactive inclusions in neurons and glial cells as revealed with several phospho-specific anti-tau antibodies (**A**–**E**) and with the phosphorylation-independent tau antibody 7.51 (**F**). Note strong immunoreactivity to antibodies to phospho-tauSer202 (**A**), Ser262 (**B**), phospho-tauThr181 (**C**), Ser422 (**D**), Ser396 (**E**), and tau 7.51 (**F**). Immunohistochemical sections slightly counterstained with hematoxylin. *Bar* 10 µm

phospho-tauSer262 and phospho-tauSer422 (all from Calbiochem) were used at a dilution of 1:2,500.

Genetic studies

Tau gene exons 9, 10, 12 and 13, were mutations in FTD patients have been found previously, were amplified using specific primers derived from the 5' and 3' sequences and the polymerase chain reaction (PCR) conditions previously described [30]. Direct sequenceanalysis was performed for these exons using an automated DNA sequencer (ABI 310; Perkin Elmer/Applied Biosystems, Foster City, CA).

Results

Neuropathological findings

Case 1

Severe frontotemporal atrophy with marked enlargement of the lateral ventricles and atrophy of the perisylvian region were observed on gross examination. Coronal sections also revealed reduced white matter and marked atrophy of the corpus callosum, as well as moderate atrophy of the caudate and putamen, and thalamus (Fig. 1). The microscopical study revealed marked neuron loss in the cerebral

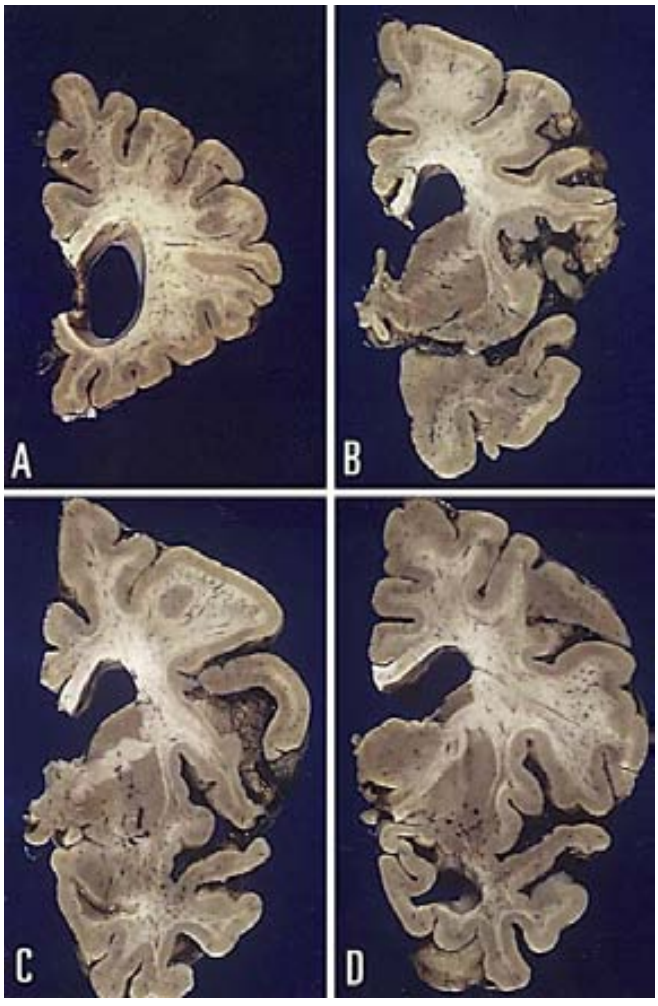


Fig. 5 Case 2. **A–D** Coronal sections of the brain. Moderate atrophy of the frontal cortex, precentral region, perisylvian region and temporal lobe, including the hippocampus. There is also moderate enlargement of the lateral ventricles, but apparent preservation of the caudate, putamen and thalamus

cortex of the frontal, temporal, insular and cingulated cortices, which was accompanied by astrocytic gliosis (Fig. 2A–C). Ballooned neurons were absent, but αB-crystallin immunohistochemistry revealed large numbers of positive reactive astrocytes (Fig. 2D). Tau-immunoreactive neurons were seen in the cerebral cortex, hippocampus, diencephalon and reticular formation (Fig. 2E–G). Pick bodies were absent. Mild neuron loss and gliosis with no tau and α-synuclein pathology was found in the substantia nigra pars compacta (Fig. 2H). Bizarre, tau-positive inclusions, resembling skein inclusions in motor neuron disease in morphology, were found in the motor nuclei of the vagus nerve (Fig. 2I).

In addition to neurons, massive accumulation of abnormal tau was present in glial cells, mainly oligodendrocytes, and in the white matter of the cerebral hemispheres, including the parietal cortex and internal capsule (Fig. 3A–D). The morphology of glial inclusions was rather globular and the inclusions were ubiquitinated (Fig. 3E). Coiled bodies were seldom observed in the cerebrum, brain stem or spinal cord (Fig. 3F). Tau deposition in neurons and glial cells was recognized with anti-phospho-specific tauThr181, Ser202, Ser214, Ser262, Ser396 and Ser422 antibodies (Fig. 4A, B, D–F). Globular glial inclusions were also strongly stained with the antibody 7.51 (Fig. 4C), but were not with anti-α-synuclein antibodies.

The neuropathological diagnosis was multiple system tauopathy.

Case 2

Atrophy of the temporal lobe, including the hippocampus, and the pre-central cortex, and mild enlargement of the lateral ventricles, were seen on gross examination (Fig. 5). Neuron loss, rare ballooned neurons and moderate astrocytic gliosis were found in the cerebral cortex; marked loss of neurons occurred in the striatum and thalamus. Abnormal tau deposition in neurons and thread-like structures in the cerebral cortex (Fig. 6A), striatum (Fig. 6B), thalamus (Fig. 6C) and brain stem (Fig. 6D) were observed. Coiled bodies in oligodendrocytes were abundant in the white matter (Fig. 6E).

Pre-tangles and grains were conspicuous in the hippocampus, entorhinal cortex, amygdala and anterior cingulate cortex (Figs. 6F, 8B). Grains were also observed in the hypothalamus. Abnormal tau deposits were stained with anti-phospho-specific tau antibodies: pre-tangles, grains and coiled bodies were stained with all the antibodies tested (Fig. 7A–C, E, F). Astrocytic plaques were clearly distinguished with anti-phospho-tauThr181, Ser202, Ser262, Ser396 and Ser 422 (Fig. 7G), but not with anti-phospho-tau Ser214 antibodies. Tufted astrocytes were absent.

Ballooned neurons in the frontal and parietal cortex were rare, although easily visualized with anti-phosphorylated neurofilament and anti-αB-crystallin antibodies (Fig. 8A). However, αB-crystallin-immunoreactive ballooned neurons were very common in the amygdala and entorhinal cortex (Fig. 8C, D).

Finally, mild neuron loss and Lewy bodies were found in the substantia nigra, locus ceruleus and brain stem reticular formation (Fig. 8E). α-Synuclein-immunoreactive dystrophic neurites were common in the lower brain stem, locus ceruleus, Meynert's nucleus and amygdala (Fig. 8F).

The neuropathological diagnosis was CBD, AGD and α-synucleinopathy.

Case 3

The gross examination revealed perisylvian atrophy and atrophy of the upper parietal and frontal cortices. There was enlargement of the lateral ventricles, reduced subcortical white matter and atrophy of the corpus callosum (Fig. 9A). The microscopical examination showed moderate neuron loss and astrocytic gliosis in the cerebral cortex. Ballooned achromatic neurons containing phosphorylated neurofilaments and αB-crystallin were found mainly in the frontal cortex, but also in the limbic system (Fig. 9B–D).

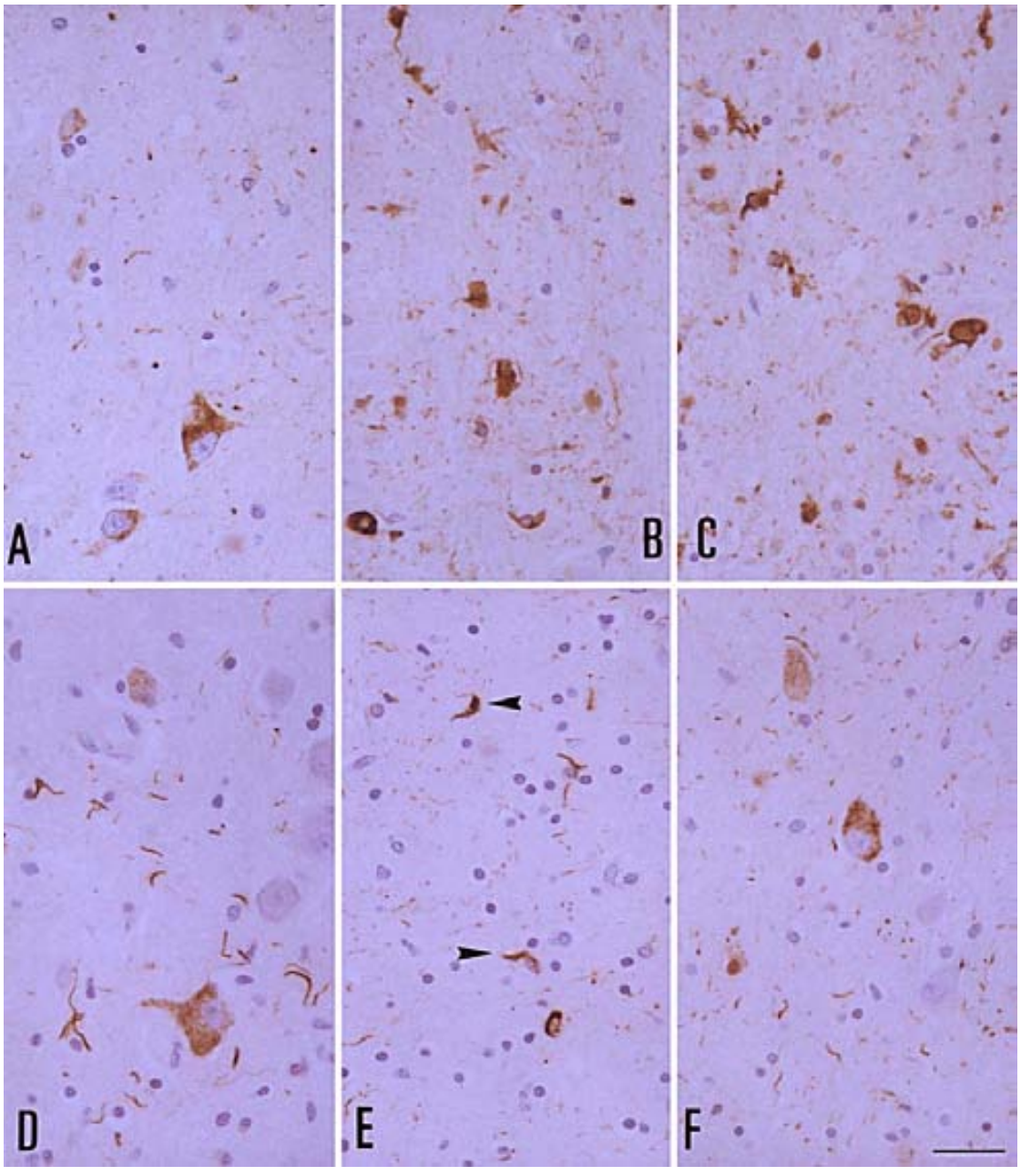


Fig. 6 Case 2. Tau-immunoreactive neurons are seen in the frontal cortex (**A**), putamen (**B**), thalamus (**C**) and pontine nuclei (**D**). Thread-like structures, although present in the cerebral cortex and diencephalon, are more easily seen in the pontine nuclei (**D**). Oligodendroglial coiled bodies are abundant in the subcortical white matter and internal capsule (**E**). In addition, neurons with pre-tangles, and numerous tau-immunoreactive grains, are observed in the hippocampus (**F**). Immunohistochemical sections slightly counterstained with hematoxylin. *Bar* 25 μm

Tau-immunoreactive neurons, although not really neurofibrillary tangles, were abundant in the cerebral cortex. However, the most striking abnormality was the presence of thread-like structures in the cerebral cortex and white matter, together with astrocytic plaques, which were visible with phospho-specific anti-tau antibodies to Thr181, Ser202, Ser396 and Ser422 (Fig. 9E). Tau-immunoreactive thread-like structures and cellular bodies, covering neurons and glial cells, were found in the striatum and thalamus (Fig. 9F, G). Moderate neuron loss was found in the substantia nigra together with corticobasal bodies that were immunoreactive with anti-phospho-specific tau antibodies. Neurofibrillary tangles (Fig. 9H, I), tau-positive glial inclusions in astrocytes and coiled bodies in oligodendrocytes were observed in the lower brain stem. βA4 amyloid and α-synuclein-immunoreactive deposits were absent.

The neuropathological diagnosis was CBD.

Case 4

Moderate cerebral atrophy was observed in the pre- and post-central region, and in the perisylvian region and temporal lobes, including the hippocampal complex. The mi-

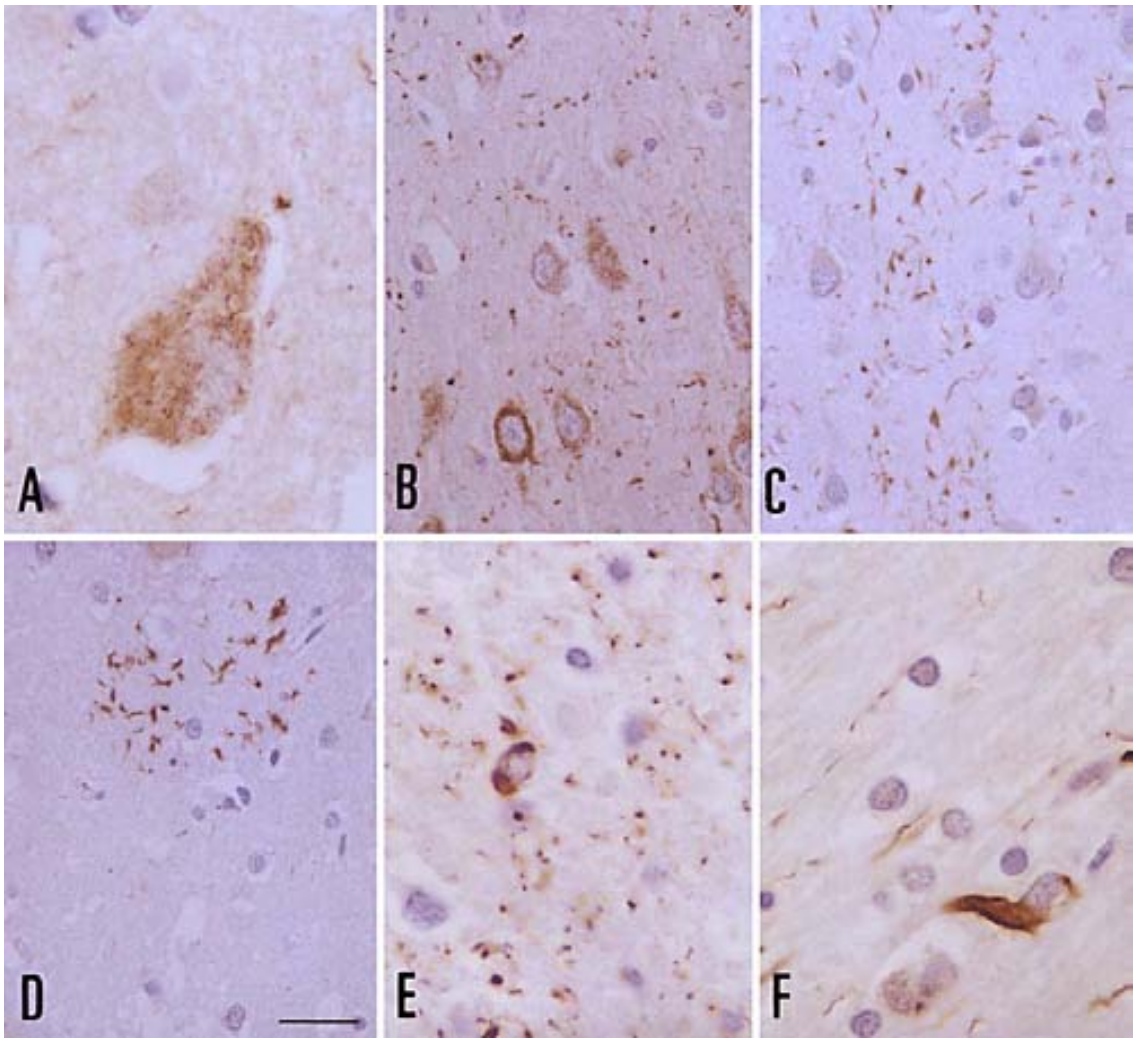


Fig. 7 Case 2. Several tau-immunoreactive deposits as seen with distinct anti-phospho-specific tau antibodies. Pre-tangle neuron in the CA1 area of the hippocampus (**A**). Pre-tangles and grains in the hippocampus (**B**). Numerous grains in the entorhinal cortex (**C**). Astrocytic plaque in the frontal cortex (**D**). Coiled bodies in the white matter (**E**) and pons (**F**). Antibodies to phospho-tauSer396 (**A**) Thr181 (**B**), Ser202 (**C**), Ser422 (**D**), Ser396, (**E**) and Ser262 (**F**). Immunohistochemical sections slightly counterstained with hematoxylin. *Bar* in **D** represents 10 µm for **A**, **F**, 25 µm for **B**–**E**

croscopical examination revealed mild neuron loss and spongiosis in the upper cortical layers of the frontal, parietal and temporal cortices, together with ballooned neurons containing phosphorylated neurofilament epitopes and αB-crystallin (Fig. 10A, B). In addition, βA4-amyloid plaques were present in the hippocampus, entorhinal areas, and neocortex (Fig. 10C) consisting of diffuse and neuritic plaques. Amyloid angiopathy affected meningeal and parenchimatous blood vessels. Neurofibrillary tangles were conspicuous in the entorhinal cortex, hippocampus and cerebral cortex. Tau-immunoreactive dystrophic neurites surrounded amyloid deposits in senile plaques (Fig. 10D). In addition, thread-like structures were abundant in the subcortical white matter and internal capsule, mainly in the vicinity of the thalamus (Fig. 10E). Thread-like structures and cellular tau-immunoreactive inclusions were common in the striatum and thalamus (Fig. 10F, G). Astrocytes and perivascular astrocytic processes in the deep temporal white matter and amygdala were decorated with anti-tau antibodies (Fig. 10H). Neurofibrillary tangles were found in the brain stem, particularly in the locus ceruleus and reticular formation. Rare α-synuclein-immunoreactive dystrophic neurites were present in the lower brain stem (Fig. 10I).

The neuropathological diagnosis was CBD, AD and α-synucleinopathy.

Western blot studies

Western blots of total brain homogenates and sarkosyl-insoluble fractions revealed particular profile patterns of phospho-tau. Cases 1–3 showed a pattern of two bands of 68 and 64 kDa in sarkosyl-insoluble fractions stained with anti-phospho-specific tau antibodies to Thr181, Ser262 and Ser422. Case 4 revealed a pattern of four bands of 73/74, 68, 64 and 60 kDa (Fig. 11).

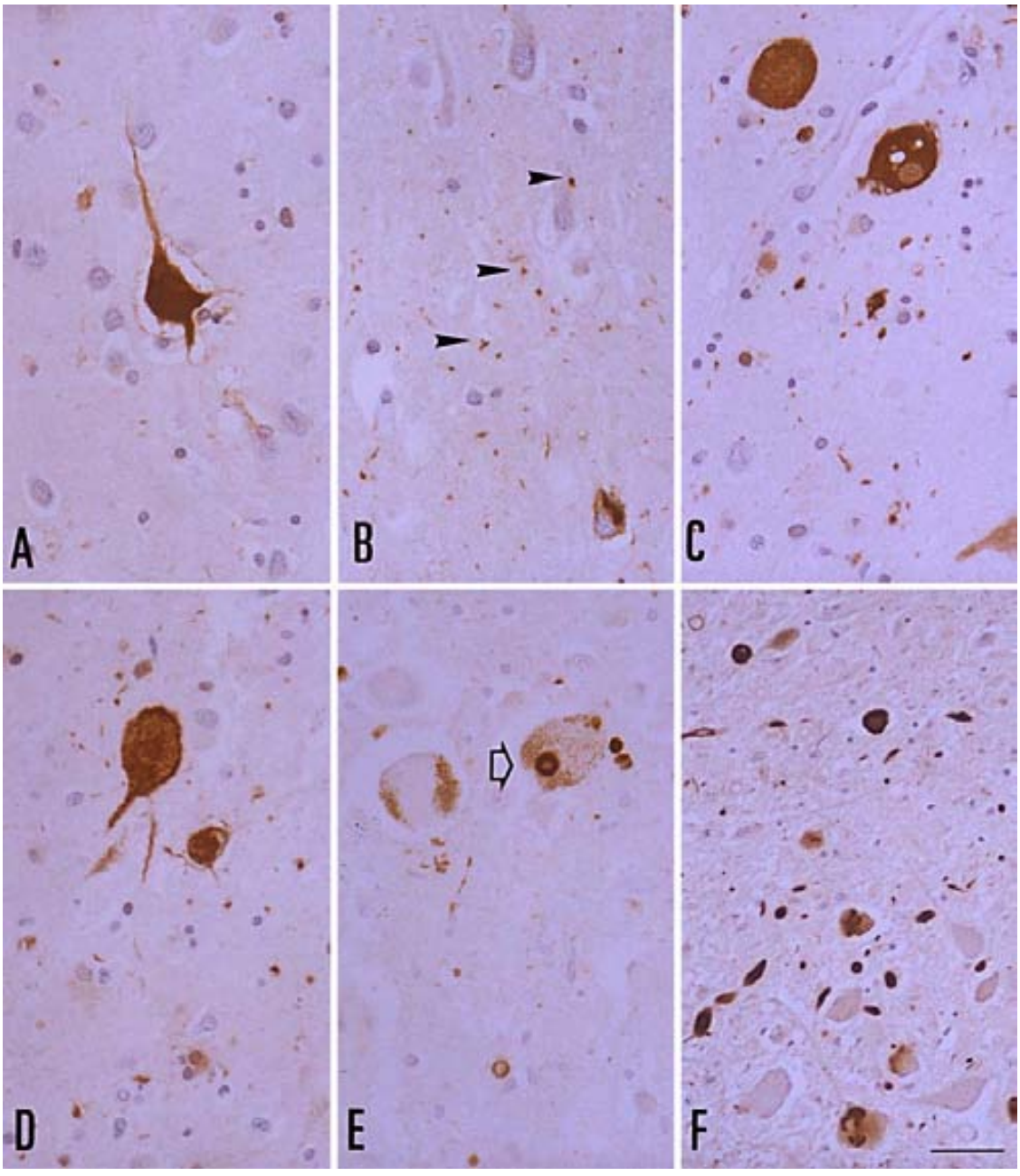


Fig. 8 Case 2. αB-crystallin-immunoreactive ballooned neuron in the frontal cortex (**A**). Tau-immunoreactive (phospho-tauSer262) grains in the CA1 area of the hippocampus (**B**). αB-crystallin-immunoreactive ballooned neurons in the amygdala (**C**) and entorhinal cortex (**D**). α-Synuclein-immunoreactive Lewy body (*white arrow*) in the Meynert's nucleus (**E**). Many α-synuclein-immunoreactive dystrophic neurites and intracytoplasmic inclusions are observed in the reticular formation of the medulla oblongata (**F**). Immunohistochemical sections slightly counterstained with hematoxylin. *Bar* 25 μm

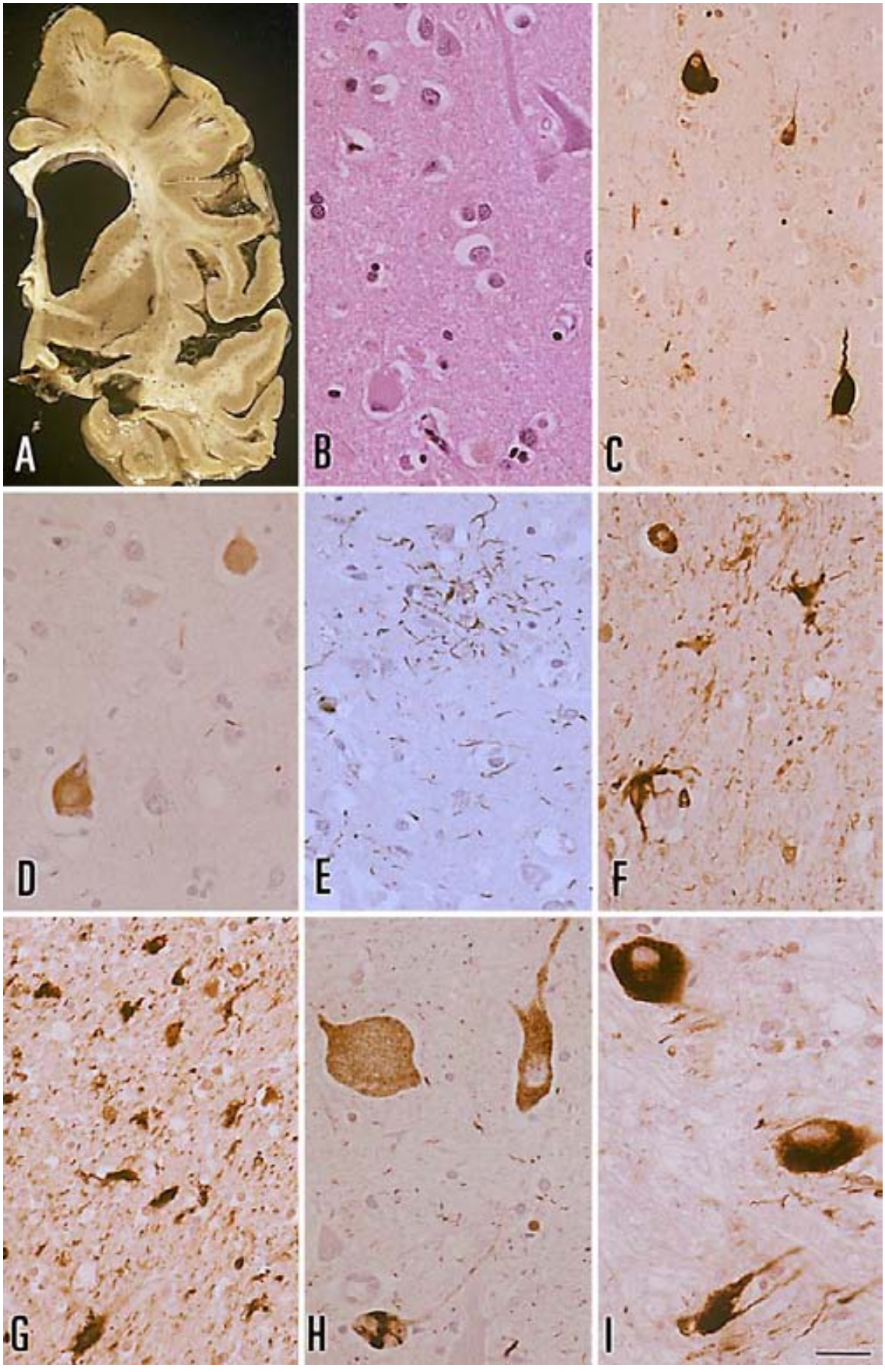
Genetic studies

Genetic studies were carried out in cases 1 and 2. No mutations in the *tau* gene were found.

Discussion

A summary of the main clinical, neuropathological, biochemical and genetic findings is given in Table 1. The initial symptoms in the four cases were expressive language

Fig. 9 Case 3. Cerebral atrophy with enlargement of the lateral ventricles (**A**). Ballooned neurons (**B**) in the cerebral cortex are immunoreactive with: anti-αB-crystallin (**C**) and anti-phosphorylated neurofilament (**D**) antibodies. Tau-immunoreactive astrocytic plaques are observed in the cerebral cortex (**E**), and tau-immunoreactive neuronal and glial inclusions are seen in the striatum (**F**) and subcortical white matter (**G**). Tau-immunoreactive neurons and neurofibrillary tangles are observed in the peri-aqueductal gray matter (**H**) and reticular formation (**I**). *Bar* **I** (also for **B**–**H**) 25 μm


A
B
C
D
E
F
G
H
I

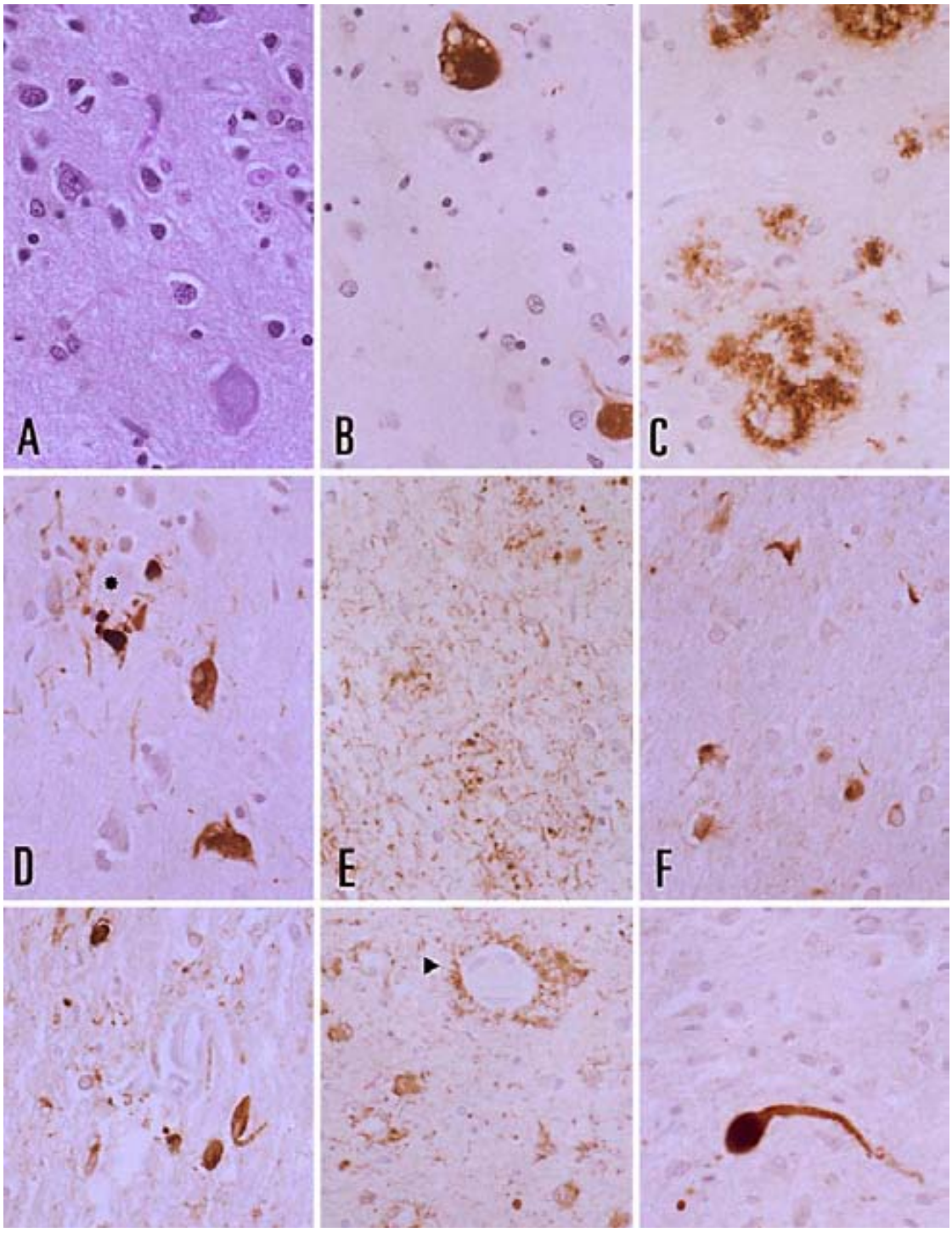


Fig. 10 Case 4. Moderate neuron loss and a ballooned neuron in the frontal cortex (**A**). Ballooned neurons are stained with anti-αB-crystallin antibodies (**B**). βA4-amyloid deposits in the cerebral cortex (**C**). Neurofibrillary tangles and dystrophic neuritis surrounding amyloid cores in neuritic plaques (*asterisk*) are stained with anti-tau antibodies (**D**). Thread-like structures are abundant in the subcortical white matter (**E**). Neurons and glial cells with abnormal tau accumulation are seen in the striatum (**F**) and thalamus (**G**). Reactive astrocytes and astroglial cell processes surrounding blood vessels (*arrowhead*) are tau immunoreactive (**H**). Isolated α-synuclein-immunoreactive profile in the reticular formation of the medulla oblongata (**I**). Immunohistochemical sections slightly counterstained with hematoxylin. *Bar* 25 μm

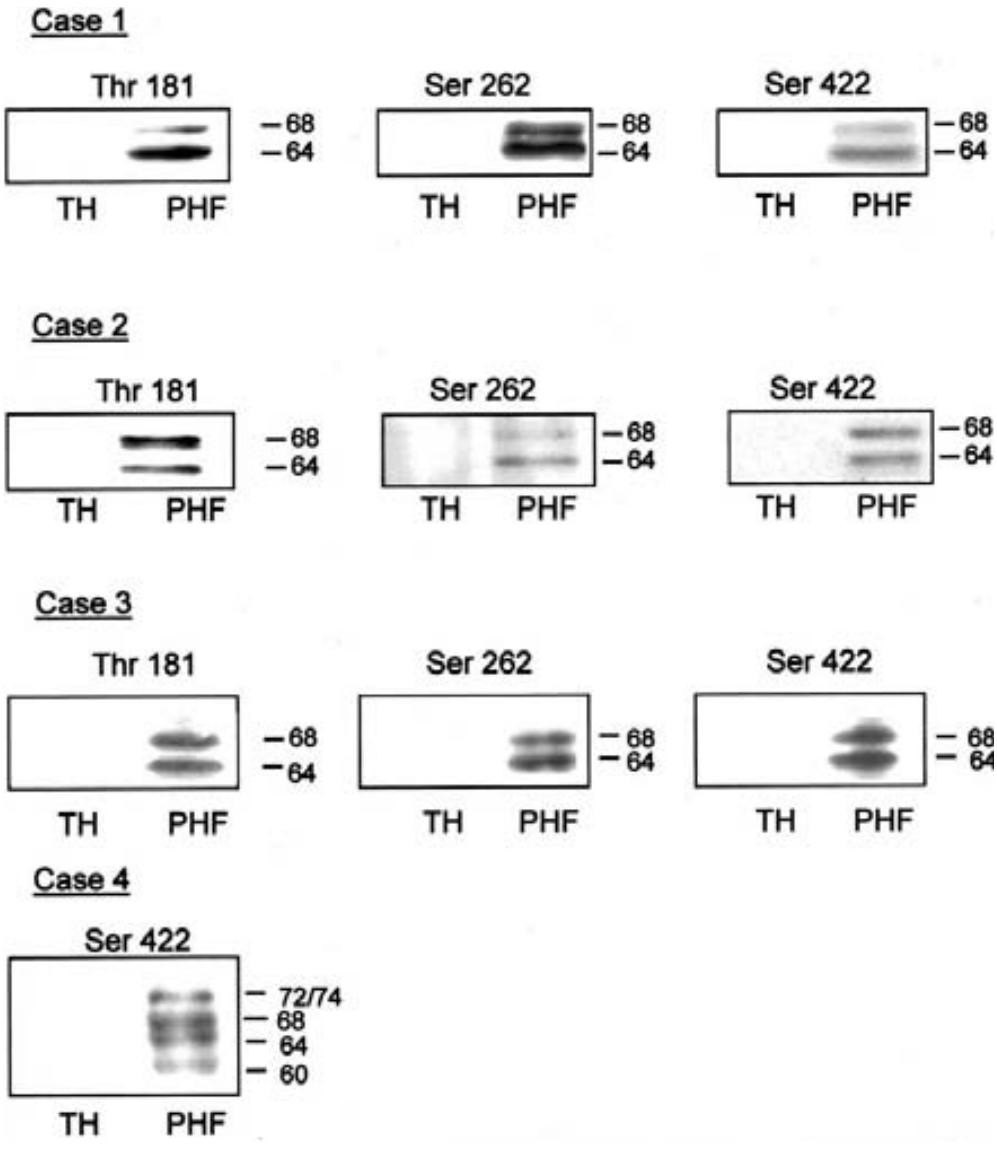


Fig. 11 Western blots of total brain homogenates (*TH*) and sarkosyl-insoluble fractions (*PHF*) in cases 1–4, processed with anti-phospho-specific tau antibodies Thr181, Ser262 and Ser422. Two bands of 68 and 64 kDa are seen in cases 1–3, whereas four bands of 72/74, 68, 64 and 60 kDa are seen in case 4

disorders, with difficulties in naming objects and articulating words. Patients 1 and 2 maintained their daily life activities up until moderately advanced stages of the disease (8 and 5 years, respectively), when the motor disorders marked the onset of their functional dependency. Speech disturbances followed by cognitive decline consistent with FTD occurred in patient 3, whereas isolated language disorder lasting for 3 years preceded rapidly progressive probable AD in case 4.

Atrophy of the superior frontal gyrus and superior parietal cortex, together with atrophy of the superior temporal gyrus, opercular area and pre-central cortex, were common, although very variable in severity, in every case of the present series. Aphasia correlated with perisylvian atrophy in each of these four cases. Atrophy of the hippocampus was marked in cases 1 and 4, but was moderate in cases 2 and 3. Interestingly, cerebral CT scans and MRI studies revealed asymmetries from the beginning of the disease in patient 1. Similarly, SPECT studies revealed progressive attenuation over time, predominating in the right side. However, in case 2, no focal signs were noticed in the SPECT studies carried out 2 years after the beginning of the speech disturbances. Unfortunately, only two cerebral CT scans showing moderate global atrophy were carried out in patients 3 and 4.

Case 1 shows rare clinical and neuropathological findings. The clinical symptoms were characterized by prolonged primary aphasia, followed by frontotemporal symptoms and, finally, by dramatic generalized cognitive devastation. The microscopical study revealed marked cortical neuronal loss with no ballooned neurons, Pick bodies, astrocytic plaques, or massive cortical neurofibrillary pathology. Yet neurons bearing abnormal phospho-tau were seen in the neocortex, diencephalons and brain stem. Moreover, large numbers of globular tau deposits were found in glial cells. Massive globular phospho-tau deposition in glial cells, as seen in case 1, has been observed in certain familial tauopathies associated with mutations and single amino acid deletions in the *tau* gene [14, 23, 34, 68] and in sporadic multiple system tauopathy with dementia [7]. Tau-

Table 1 Summary of the main clinical, neuropathological, biochemical and genetic findings in this series. α-synucleinopathy indicates Lewy body disease with brain stem involvement (Parkinson's disease), Western blot tau: indicates the pattern of bands of phospho-*tau* in sarkosyl-insoluble fractions (*PPA* primary progressive aphasia, *FTD* frontotemporal dementia, *ND* not done, *CBD* corticobasal degeneration, *AGD* argyrophilic grain disease, *AD* Alzheimer's disease)

Case	Age at onset (years)	Gender	First symptoms	Course	Familial antecedents	Age at death	Genetic study	Western blot tau	Neuropathological diagnosis
1	66	F	PPA	FTD	No	81		68, 64	Multiple system tauopathy
2	69	F	PPA	FTD + asymmetric rigidity	No	78		68, 64	CBD+ AGD + α-synucleinopathy
3	66	M	PPA	CBD syndrome	No	73	ND	68, 64	CBD
4	68	M	PPA	Dementia	No	73	ND	74, 68, 64, 60	CBD + AD + α-synucleinopathy

immunoreactive glial inclusions in those cases and in the present case differ from glial inclusions in multiple system atrophy (MSA), although both are ubiquitinated. Glial inclusions in MSA contain α-synuclein and non-phosphorylated tau [2, 13, 18, 46, 69, 76, 80], whereas glial inclusions in the present case were negative for α-synuclein but strongly immunoreactive for phosphorylated tau and with the phosphorylation-independent tau antibody 7.51.

Case 2 showed neuropathological findings of CBD, AGD and α-synucleinopathy. A diagnosis of CBD was based on the presence of tau-immunoreactive inclusions in neurons and glial cells in the striatum, thalamus, cerebral cortex and selected nuclei of the brain stem, thread-like structures in the diencephalon, white matter and pontine nuclei, and astrocytic plaques in the cerebral cortex. There was a paucity of ballooned neurons in the frontal cortex, but this does not exclude a diagnosis of CBD [16]. In addition, the diagnosis of AGD was based on the presence of pre-tangles and tau-immunoreactive grains in the anterior and posterior hippocampus, entorhinal cortex, amygdala, anterior gyrus cinguli and hypothalamus [8, 35, 71, 74]. Ballooned neurons containing αB-crystallin in the amygdala and entorhinal cortex, and oligodendroglial coiled bodies, as seen in this case, are common in CBD and AGD [72]. However, the large number of ballooned neurons in the amygdala, in striking contrast with the limited number of such neurons in the frontal cortex, is likely due to AGD rather than to CBD.

AGD has been reported in combination with AD, dementia with tangles, PSP and several α-synucleinopathies [8, 35, 52]. Argyrophilic grains were found in 2 cases of CBD in a series of 16 incidental cases [52] and in 2 of 5 cases of CBD in our files at the Barcelona and Bellvitge Banks of Nervous and Muscular Tissues (BBB) (unpublished observations). Lewy bodies (LBs) and α-synuclein dystrophic neurites in select nuclei of the brain stem, Meynert's nuclei and amygdala were observed in the present case. Co-occurrence of PD with PSP has been documented [37, 57]. The combination of PD or diffuse Lewy body disease and tauopathy in the same patient is not exceptional. LBs were found in 3 of 13 cases of PSP, 2 of 5 cases of CBD and 2 cases with combined PSP and CBD in our files at the BBB (unpublished observations). Primary progressive aphasia has never been reported in AGD, and it is probably a very rare symptom in this disease, mainly due to the restricted limbic distribution of the lesions in AGD, as in the present case. Therefore, primary aphasia can be ascribed, rather, to CBD. However, cognitive impairment appearing late in the course of the disease was probably due to concomitant AGD, as cortical abnormalities, excepting those consistent with limbic AGD, were scanty in this particular case. Asymmetrical rigidity appeared during the last year of the disease, but the clinical relevance of PD changes versus CBD-like striatal pathology in this patient remains obscure.

Case 3 of the present series had typical clinical and neuropathological features of CBD [4, 5, 16, 19, 39, 49, 63]. These included ballooned neurons, immunoreactive to αB-crystallin and phosphorylated neurofilaments, in the frontal and parietal cortex, and astrocytic plaques in the cerebral cortex. Constant accompanying features were scattered cortical neurons and large numbers of cell processes (thread-like structures) containing phosphorylated tau in the cerebral cortex, white matter and diencephalic nuclei. Tau-containing neurons in the locus ceruleus and other areas of the brain stem often resembling corticobasal bodies in monoaminergic nuclei were also observed. All these characteristics have been well documented in CBD [16, 17, 19, 20, 44, 50, 70, 78, 79]. Coiled bodies in oligodendrocytes, as seen in case 3, are present in CBD, but also in PiD, PSP and AGD [16, 19, 21, 31, 44]. Primary progressive aphasia has been recognized as a possible initial symptom in CBD [1, 4, 5, 45, 55, 62, 83]. Case 3 lends further support to the hypothesis that CBD may start with primary progressive aphasia followed by cognitive impairment.

Case 4 had combined findings of AD and CBD, as well as rare α-synuclein inclusions in the lower brain stem. Neurofibrillary pathology corresponding to stage VI of Braak and Braak, and βA4 amyloid deposition stage C, define advanced AD [10]. Yet massive thread-like structures in the cerebral white matter, αB-crystallin-immuno-

reactive ballooned neurons in the frontal cortex, and abundant tau-positive inclusions in the striatum and thalamus, even in the absence of clear cortical astrocytic plaques possibly masked by neuritic plaques, were consistent with CBD. Such a combination is not strange due to the prevalence and increased risk of AD in the elderly [16]. However, this is the first description of co-occurrence of AD and CBD in primary progressive aphasia. It is worth mentioning that primary progressive aphasia has also been reported as the initial symptom in AD [3, 28, 37, 60]. Finally, discrete α-synuclein inclusions and dystrophic neurites in the lower brain stem, as seen in this case, have also been noted as a pre-clinical state in PD [14].

The biochemical patterns of phosphorylated tau in total homogenates and sarkosyl-insoluble fractions have shown four bands of 73/74, 68, 64 and 60 kDa of phospho-tau in sarkosyl-insoluble fractions in case 4, indicating the predominance of AD-like phosphorylated tau deposition [11, 12, 47]. Two phospho-tau bands of 68 and 64 kDa occurred in cases 1–3. This pattern is similar in CBD [11, 47].

Taken together, these observations from the present series demonstrate that primary progressive aphasia may occur in distinct tauopathies and that CBD, or CBD combined with other tauopathies, is a main cause of primary progressive aphasia. Based on similar findings, other authors have proposed including all these diseases under the term frontotemporal degeneration or Pick complex [38]. These terms are useful operational tools in the current clinical practice, but help little to increase our understanding of the morphological, biochemical and genetic diversity that is under the term Pick complex. In contrast, the present message is focused on favoring the concept that complete clinical and neuropathological examination, together with biochemical and genetic studies, are essential to delineate old and new tauopathies manifested as primary progressive aphasia.

Acknowledgements This work was supported in part by EU contract QLRI-CT-2000-66, CICYT SAF-2001-4681-E and FIS P1020004. We greatly acknowledge Dr. J. Avila his comments and criticism. We wish to thank M. Barrachina for help with the figures and T. Yohannan for editorial assistance.

References

1. Arima K, Uesugi H, Fujita I, Sakurai Y, Oyanagi S, Andoh S, Izumiyama Y, Inose T (1994) Corticonigral degeneration with neuronal achromasia presenting with primary progressive aphasia: ultrastructural and immunocytochemical studies. J Neurol Sci 127:186–197
2. Arima K, Ueda K, Sunohara N, Arakawa K, Hirai S, Nakamura M, Tonozuka-Uehara H, Kawai M (1998) NACP/α-synuclein immunoreactivity in fibrillary components of neuronal and oligodendroglial cytoplasmic inclusions in the pontine nuclei in multiple system atrophy. Acta Neuropathol 96:439–444
3. Benson DF, Zaias BW (1991) Progressive aphasia: a case with post-mortem correlation. Neuropsychiatr Neuropsylchol Behav Neurol 4:215–223
4. Bergeron C, Pollanen M, Weyer L, Black S, Lang A (1996) Unusual clinical presentation of cortical-basal ganglionic degeneration. Ann Neurol 40:893–900
5. Bergeron C, Davis A, Lang AE (1998) Corticobasal ganglionic degeneration and progressive supranuclear palsy presenting with cognitive decline. Brain Pathol 8:355–365
6. Bigio EH, Lipton AM, Yen SH, Hutton ML, Baker M, Nacharaju P, White ChL, Davies P, Lin W, Dickson DW (2001) Frontal lobe dementia with novel tauopathy: sporadic multiple system tauopathy with dementia. J Neuropathol Exp Neurol 60: 328–341
7. Boeve B, Dickson D, Duffy J, Bartleson J, Trenerry M, Petersen R (2003) Progressive nonfluent aphasia and subsequent aphasic dementia associated with atypical progressive supranuclear palsy. Eur Neurol 49:72–78
8. Braak H, Braak E (1989) Cortical and subcortical argyrophilic grains characterize a disease associated with adult onset dementia. Neuropathol Appl Neurobiol 15:13–26
9. Braak H, Braak E (1998) Argyrophilic grain disease: frequency of occurrence in different age categories and neuropathological diagnostic criteria. J Neural Transm 105:801–819
10. Braak H, Braak E (1999) Temporal sequence of Alzheimer's disease-related pathology. Cereb Cortex 14:475–512
11. Buée L, Delacourte A (1999) Comparative biochemistry of tau in progressive supranuclear palsy, corticobasal degeneration, FTDP-17 and Pick's disease. Brain Pathol 9:681–693
12. Buée L, Bussière T, Buée-Scherrer V, Delacourte A, Hof PR (2000) Tau isoforms, phosphorylation and role in neurodegenerative disorders. Brain Res Rev 33:95–130
13. Burn DJ, Jaros E (2001) Multiple system atrophy: cellular and molecular pathology. J Clin Pathol: Mol Pathol 54:419–426
14. Del Tredici K, Rüb U, Vos RAI de, Bohl JRE, Braak H (2002) Where does Parkinson disease pathology begin in the brain? J Neuropathol Exp Neurol 61:413–426
15. Delisle MB, Murrell JR, Richardson R, Trofatter JA, Rascol O, Soulages X, Mohr M, Calvas P, Ghetti B (1999) A mutation at codon 279 (N279 K) in exon 10 of the tau gene causes tauopathy with dementia and supranuclear palsy. Acta Neuropathol 98:62–77
16. Dickson DW (2002) Progressive supranuclear palsy and corticobasal degeneration. In: Hof PR, Mobbs CV (eds) Functional neurobiology of aging. Academic Press, San Diego, pp 165–171
17. Dickson DW, Yen SH, Suzuki KI, Davies P, Garcia JH, Hirano A (1986) Ballooned neurons in select neurodegenerative diseases contain phosphorylated neurofilament epitopes. Acta Neuropathol 71:216–223
18. Dickson DW, Lin WL, Liu WK, Yen SH (1999) Multiple system atrophy: a sporadic synucleinopathy. Brain Pathol 9:721–732
19. Dickson DW, Bergeron C, Chin SS, Duyckaerts C, Horoupian D, Ikeda K, Jellinger K, Lantos PL, Lippa CF, Mirra SS, Tabaton M, Vonsattel JP, Wakabayashi K, Litvan I (2002) Office of rare diseases neuropathologic criteria for corticobasal degeneration. J Neuropathol Exp Neurol 61:935–946
20. Feany MB, Dickson DW (1995) Widespread cytoskeletal pathology characterizes corticobasal degeneration. Am J Pathol 146:1388–1396
21. Feany MB, Dickson DW (1996) Neurodegenerative disorders with extensive tau pathology: a comparative study and review. Ann Neurol 40:139–148
22. Ferrer I, Barrachina M, Tolnay M, Rey MJ, Vidal N, Carmona M, Blanco R, Puig B (2003) Phosphorylated protein kinases associated with neuronal and glial tau deposits in argyrophylic grain disease. Brain Pathol 13:62–78
23. Ferrer I, Pastor P, Rey MJ, Muñoz E, Puig B, Pastor E, Oliva R, Tolosa E (2003) Tau phosphorylation and kinase activation in familial tauopathy linked to delN296 mutation. Neuropathol Appl Neurobiol 29:23–34
24. Gibb WRG, Luthert PJ, Marsden CD (1989) Corticobasal degeneration. Brain 116:281–302
25. Goedert M, Spillantini MG, Cairns NJ, Crowtler RA (1992) Tau proteins of Alzheimer paired helical filaments: abnormal phosphorylation of all six brain isoforms. Neuron 8:159–168

26. Goedert M, Spillantini MG, Davis SW (1998) Filamentous nerve cell inclusions in neurodegenerative diseases. Curr Opin Neurobiol 8:619–632
27. Graff-Radford NR, Damasio AR, Hyman BT, Hart MN, Tranel D, Damasio H, Van Hoesen GW (1990) Progressive aphasia in a patient with Pick's disease: neuropsychological, radiologic, and anatomic study. Neurology 40:620–626
28. Green J, Moris JC, Sandson J, McKeel DW, Miller JW (1990) Progressive aphasia: a precursor of global dementia? Neurology 40:423–429
29. Holland AL, McBurney DH, Moossy J, Reinmuth OM (1985) The dissolution of language in Pick's disease with neurofibrillary tangles: a case study. Brain Lang 24:36–58
30. Hutton M, Lendon CL, Rizzu P, et al (1996) Association of a missense and 5'splice-site mutations in tau with the inherited dementia FTDP-17. Nature 393:702–705
31. Ikeda K, Akiyama H, Kiondo H, Haga C (1995) A study of dementia with argyrophilic grains. Possible cytoskeletal abnormality in dendrospinal portion of neurons and oligodendroglia. Acta Neuropathol 89:409–414
32. Ikeda K, Akiyama H, Iritani S, Kase K, Arai T, Niizato K, Kuroki N, Kosaka K (1996) Corticobasal degeneration with primary progressive aphasia and accentuated cortical lesion in superior temporal gyrus: case report and review. Acta Neuropathol 92:534–539
33. Ingram EM, Spillantini MG (2002) Tau gene mutations: dissecting the pathogenesis of FTDP-17. Trends Mol Med 8:555–562
34. Iseki E, Matsumura T, Marui W, Hino H, Odawara T, Sugiyama N, Suzuki K, Sawada H, Arai T, Kosaka K (2001) Familial frontotemporal dementia and parkinsonism with a novel N296H mutation in exon 10 of the *tau* gene and a widespread tau accumulation in the glial cells. Acta Neuropathol 102:285–292
35. Jellinger KA (1998) Dementia with grains (argyrophilic grain disease). Brain Pathol 8:377–386
36. Judkins AR, Forman MS, Uryu K, Hinkle DA, Asbury AK, Lee VMY, Trojanowski JQ (2002) Co-occurrence of Parkinson's disease with progressive supranuclear palsy. Acta Neuropathol 103:526–530
37. Kempler D, Metter EJ, Riege WH, Jackson CA, Benson DF, Hanson WR (1990) Slowly progressive aphasia: three cases with language, memory, CT and PET data. J Neurol Neurosurg Psychiatry 53:987–993
38. Kertesz A, Muñoz DG (2003) Primary progressive aphasia and Pick complex. J Neurol Sci 206:97–107
39. Kertesz A, Hudson L, Mackenzie IR, Muñoz DG (1994) The pathology and nosology of primary progressive aphasia. Neurology 44:2065–2072
40. Kertesz A, Martínez-Lage P, Davidson W, Muñoz DG (2000) The corticobasal degeneration syndrome overlaps progressive aphasia and frontotemporal dementia. Neurology 55:1368–1375
41. Kinoshita A, Tomimoto H, Tachibana N, Suenaga T, Kawamata T, Kimura T, Akiguchi I, Kimura J (1996) A case of primary progressive aphasia with abnormally ubiquitinated neurites in the cerebral cortex. Acta Neuropathol 92:520–524
42. Kirschner HS, Tanridag O, Thurman L, Whetsell WO (1987) Progressive aphasia without dementia: two cases with spongiform degeneration. Ann Neurol 22:527–532
43. Knopman DS (1993) Overview of dementia lacking distinctive histology: pathological designation of a progressive dementia. Dementia 4:132–136
44. Komori T (1999) Tau-positive glial inclusions in progressive supranuclear palsy, corticobasal degeneration and Pick's disease. Brain Pathol 9:663–679
45. Lang AE (1992) Cortical basal ganglionic degeneration presenting with progressive loss of speech output and orofacial dyspraxia. J Neurol Neurosurg Psychiatry 55:1101
46. Lantos PL (1998) The definition of multiple system atrophy: a review of recent developments. J Neuropathol Exp Neurol 57: 1099–1111
47. Lee VMY, Goedert M, Trojanowski JQ (2001) Neurodegenerative tauopathies. Annu Rev Neurosci 24:1121–1159
48. Lippa CF, Cohen R, Smith TW, Drachman DA (1991) Primary progressive aphasia with focal neuronal achromasia. Neurology 41:882–886
49. Litvan I, Grimes DA, Lang AE (2000) Phenotypes and prognosis: clinicopathological studies of corticobasal degeneration. Adv Neurol 82:183–196
50. Lowe J, Errington DR, Lennox G, Pike I, Spendlove I, Landon M, Mayer RJ (1992) Ballooned neurons in several neurodegenerative diseases and stroke contain αB-crystallin. Neuropathol Appl Neurobiol 18:341–350
51. Mandell AM, Alexander MP, Carpenter S (1989) Creutzfeldt-Jakob disease presenting as isolated aphasia. Neurology 39:55–58
52. Martinez-Lage P, Muñoz DG (1997) Prevalence of diseases associated with argyrophilic grains of Braak. J Neuropathol Exp Neurol 56:157–164
53. Mesulam MM (1982) Slowly progressive aphasia without generalized dementia. Ann Neurol 11:592–598
54. Mesulam MM (1987) Primary progressive aphasia – differentiation from Alzheimer's disease. Ann Neurol 22:533–534
55. Mimura M, Oda T, Tsuchiya K, Kato M, Ikeda K, Hori K, Kashima H (2001) Corticobasal degeneration presenting with nonfluent primary progressive aphasia: a clinicopathological study. J Neurol Sci183:19–26
56. Mochizuki A, Ueda Y, Komatsuzaki Y, Tsuchiya K, Arai T, Shoji S (2003) Progressive supranuclear palsy presenting with primary progressive aphasia. Clinicopathological report of an autopsy. Acta Neuropathol 105:610–614
57. Mori H, Yoshimura M, Tomonaga M, Yamanouchi H (1986) Progressive supranuclear palsy with Lewy bodies. Acta Neuropathol 71:344–346
58. Neary D, Snowden JS, Mann DM (1993) Familial progressive aphasia: its relationship to other forms of lobar atrophy. J Neurol Neurosurg Psychiatry 56:1122–1125
59. Novak M, Jakes PC, Edwards C, Milstein C, Wischik CM (1991) Difference between the tau protein of Alzheimer paired helical filament core and normal tau revealed by epitope analysis of monoclonal antibodies 423 and 7.51. Proc Natl Acad Sci USA 88:5837–5841
60. Pogakar S, Williams RS (1984) Alzheimer's disease presenting as slowly progressive aphasia. RI Med J 67:181–185
61. Rebeiz JJ, Kolodny EH, Richardson EP (1968) Corticodentatonigral degeneration with neuronal achromasia. Arch Neurol 18:20–33
62. Sakurai Y, Hashida H, Uesugi H, Arima K, Murayama S, Bando M, Iwata M, Momose T, Sakuta M (1996) A clinical profile of corticobasal degeneration presenting as primary progressive aphasia. Eur Neurol 36:134-137
63. Scheltens P, David R, Kampshorst W (1994) Pathologic findings in a case of primary progressive aphasia. Neurology 44: 279–282
64. Schneider JA, Watts RL, Gearing M, Brewer RP, Mirra SS (1997) Corticobasal degeneration: neuropathological and clinical heterogeneity. Neurology 48:959–969
65. Schwarz M, De Blesser R, Poeck K, Weis J (1998) A case of primary progressive aphasia. A 14-year follow-up study with neuropathological findings. Brain 121:115–126
66. Shuttleworth EC, Yates AJ, Paltan-Ortiz JD (1985) Creutzfeldt-Jakob disease presenting as progressive aphasia. J Natl Med Assoc 77:649–655
67. Snowden JS, Neary D, Mann DMA, Goulding PJ, Testa HJ (1992) Progressive language disorder due to lobar atrophy. Ann Neurol 31:174–183
68. Spillantini MG, Goedert M, Crowther RA, Murrell JR, Farlow MR, Ghetti B (1997) Familial multiple system tauopathy with presenile dementia: a disease with abundant neuronal and glial tau filaments. Proc Natl Acad Sci USA 94:4113–4118

69. Spillantini MG, Crowther RA, Jakes R, Cairns NJ, Lantos PL, Goedert M (1998) Filamentous α-synuclein inclusions link multiple system atrophy with Parkinson's disease and dementia with Lewy bodies. Neurosci Lett 251:205–208
70. Takahashi T, Amano N, Hanihara T, Nagatiomo H, Yagishita S, Itoh Y, Yamaoka K, Toda H, Tanabe T (1996) Corticobasal degeneration: widespread argentophilic threads and glia in addition to neurofibrillary tangles. Similarities of cytoskeletal abnormalities in corticobasal degeneration and progressive supranuclear palsy. J Neurol Sci 138:66–77
71. Togo T, Cookson N, Dickson DW (2001) Argyrophilic grain disease: neuropathology. Frequency in a dementia brain bank and lack of relationship with apolipoprotein E. Brain Pathol 12: 45–52
72. Togo T, Sahara N, Yen SH, Cookson N, Ishizawa T, Hutton M, De Silva R, Lees A, Dickson DW (2002) Argyrophilic grain disease is a sporadic 4-repeat tauopathy. J Neuropathol Exp Neurol 61:547–556
73. Tolnay M, Probst A (1998) Ballooned neurons expressing α-B-crystallin as a constant feature of the amygdala in argyrophilic grain disease. Neurosci Lett 246:165–168
74. Tolnay M, Schwietert M, Monsch AU, Stahelin HB, Langui D, Probst A (1997) Argyrophilic grain disease: distribution of grains in patients with and without dementia. Acta Neuropathol 94: 353–358
75. Tolnay M, Sergeant N, Ghestem A, Chalbot S, Vos RAI de, Jansen Steur ENH, Probst A, Delacourte A (2002) Argyrophylic grain disease and Alzheimer's disease are distinguished by their different distribution of tau protein isoforms. Acta Neuropathol 104:425–434
76. Tu PH, Galvin JE, Baba M, Giasson B, Tomita T, Leigh S, Nakajo S, Iwatsuboto T, Trojanowski JQ, Lee VMY (1998) Glial cytoplasmic inclusions in white matter oligodendrocytes of multiple system atrophy brains contain insoluble α-synuclein. Ann Neurol 44:415–422
77. Turner RS, Kenyon LC, Trojanowski JQ, Gonatas N, Grossman M (1996) Clinical, neuroimaging, and pathologic features of progressive nonfluent aphasia. Ann Neurol 39:166–173
78. Uchihara T, Mitani K, Mori H, Kondo H, Yamada M, Ikeda K (1994) Abnormal cytoskeletal pathology peculiar to corticobasal degeneration is different from that of Alzheimer's disease or progressive supranuclear palsy. Acta Neuropathol 88:379–383
79. Wakabayashi K, Oyanagi K, Makifuchi T, Ikuta F, Honna A, Honna Y, Horikawa Y, Tokiguche S (1994) Corticobasal degeneration. Etiopathological significance of the cytoskeletal alterations. Acta Neuropathol 87:545–553
80. Wakabayashi K, Hayashi S, Kakita A, Yamada M, Toyoshima Y, Yoshimoto M, Takahashi H (1998) Accumulation of α-synuclein/NACP is a cytopathological feature common to Lewy body disease and multiple system atrophy. Acta Neuropathol 96:445–452
81. Wechsler AF, Verity MA, Rosenschein S, Fried I, Scheibel AB (1982) Pick's disease. A clinical, computed tomographic, and pathological study with Golgi impregnation observations. Arch Neurol 39:287–290
82. Yamanouchi H, Budka H, Vass K (1986) Unilateral Creutzfeldt-Jakob disease. Neurology 36:1517–1520
83. Yoshimura I, Yoshimura N, Hayashi S, Tasaki H, Kukushima Y (1995) A clinicopathological study of senile dementia presenting as progressive sensory aphasia: a variant of corticobasal degeneration. Jpn J Clin Psychiatry 24:193–200
84. Zhukareva V, Vogelsberg-Ragaglia V, Van Deerlin VMD, Bruce J, Shuck T, Grossman M, Clark CM, Arnold SE, Masliah E, Galasko D, Trojanowski JQ, Lee VMY (2001) Loss of brain tau defines novel sporadic and familial tauopathies with frontotemporal dementia. Ann Neurol 49:165–175