Enfermedad de Alzheimer de inicio presenil esporádica: Caracterización clínico-biológica y diagnóstico precoz

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La Dra. Raquel Sánchez del Valle Díaz, doctora en Medicina por la Universidad de Barcelona y el Dr. Albert Lladó Plarrumaní, doctor en Medicina por la Universidad de Barcelona,

CERTIFICAN:

Que la memoria titulada *"Enfermedad de Alzheimer de inicio presenil esporádica: caracterización clínico-biológica y diagnóstico precoz"*, presentada por Mircea Balasa, ha estado realizada bajo nuestra dirección y consideramos que reúne las condiciones necesarias para ser defendida ante el Tribunal correspondiente para optar al grado de Doctor por la Universidad de Barcelona.

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Pe când nu era moarte, nimic nemuritor,
Nici sâmburul luminii de viatã dãtãtor,
Nu era azi, nici mâine, nici ieri, nici totdeauna,
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Pe când pãmântul, cerul, vãzduhul, lumea toatã
Erau din rândul celor ce n-au fost niciodatã,
Pe-atunci erai Tu singur, încât mã-ntreb în sine-mi:
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ÍNDICE

I.

LISTADO DE ABREVIATURAS

Aβ 1-42 Isoforma de 42 aminoácidos de la proteína beta-amiloide

ADL Actividades de la vida diaria (Activities of daily living)

APP Gen de la proteína precursora del amiloide (Amyloid precursor protein)

APOE Gen de la apolipoproteina E

BTN Banco de tejidos neurológicos

CTh Grosor cortical (Cortical thickness)

DCL Deterioro cognitivo leve

DSM Diagnostic and Statistical Manual of Mental Disorders

EA Enfermedad de Alzheimer

EAP Enfermedad de Alzheimer de inicio precoz

EAT Enfermedad de Alzheimer de inicio tardío

ELISA Enzyme-linked immuno sorbent assay

HAD Herencia autosómica dominante

LCR Líquido cefalorraquídeo

MMSE Mini Mental State Examination

NIA-AA National Institute of Aging- Alzheimer's Association

NINCDS-ADRDA National Institute of Neurologic, Communicative Disorders and Stroke - Alzheimer's disease and Related Disorders Association

PET Tomografía por emisión de positrones

FDG PET PET cerebral con el trazador [^{18}F]-fluorodeoxiglucosa

PICOGEN Programa de información y consejo genético para demencias familiares del Hospital Clínic de Barcelona

PSEN1 Gen presenilina 1

PSEN2 Gen presenilina 2

RM Resonancia magnética

SPSS Statistical Product and Service Solutions

UATC Unidad de Alzheimer y otros trastornos cognitivos, Hospital Clínic de Barcelona

II.

INTRODUCCIÓN

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1. Enfermedad de Alzheimer de inicio precoz y tardío: epidemiología, clínica y diagnóstico

La Enfermedad de Alzheimer (EA) es la demencia neurodegenerativa más frecuente, con una prevalencia mundial estimada de unos 35 millones de casos. Sin embargo, el previsible aumento de la esperanza de vida aumentará de forma exponencial su prevalencia en las próximas décadas, pudiendo afectar unos 80 millones de personas en 2040, con el consiguiente aumento de la carga asistencial y del gasto sanitario (Ferri et al., 2005; Alzheimer's Disease International Consortium, 2009).

Según la edad de inicio de los síntomas la EA se ha dividido de forma arbitraria en dos formas clínicas: EA de inicio precoz o presenil (EAP) cuando la enfermedad se manifiesta clínicamente antes de los 65 años y EA de inicio tardío (EAT) cuando los primeros síntomas aparecen por encima de los 65 años. El principal factor de riesgo de la EA es la edad, con tasas de incidencia que se duplican cada 5 años a partir de los 65 años. A pesar de eso, aproximadamente el 10% de los casos de EA se inician antes de los 65 años, siendo la EA la demencia de inicio precoz más frecuente en nuestro entorno, al describirse prevalencias entre 30-60% del total de las demencias de inicio precoz (Harvey et al., 2003; Garre-Olmo et al., 2010).

La primera descripción de lo que posteriormente sería conocido como EA, realizada por Alois Alzheimer en 1907, fue la de una paciente con una EAP: Auguste D inició a los 50 años un cuadro de alteración mnésica y conductual que progresó rápidamente durante los años posteriores falleciendo a los 56 años. El examen neuropatológico de su cerebro mostró por primera vez los hallazgos característicos de la EA, es decir, las placas seniles y neuríticas y los ovillos neurofibrilares (Alzheimer, 1907). El término enfermedad de Alzheimer, que inicialmente se utilizó para designar a la EA de inicio precoz se generalizó posteriormente a todos los casos de demencia de naturaleza degenerativa y curso progresivo que comparten este substrato patológico, independientemente de la edad de inicio.

Durante décadas la EA ha sido una entidad clínico-patológica. Así, para poder realizar un diagnóstico de certeza de EA el paciente debía presentar un deterioro cognitivo suficientemente importante como para causar demencia, a la vez que debía demostrarse la presencia de las lesiones características de la EA en el examen patológico. La demencia según los criterios DSM IV o ICD-10 (World Health Organization - Classification of Mental and Behavioural Disorders, 1993) es definida como un déficit cognitivo que causa una

alteración significativa en el funcionamiento social o laboral y representa un claro declive respecto a la situación basal del sujeto. En este contexto, el diagnóstico clínico en vida de la EA según los criterios diagnósticos NINCDS-ADRDA (McKhan et al., 1984), es sólo un diagnóstico de probabilidad que requiere la presencia de demencia con afectación de varios dominios cognitivos (uno de los cuales ha de ser la memoria episódica), un inicio insidioso, un curso progresivo, y haber descartado otras etiologías posibles de deterioro cognitivo. Las pruebas complementarias, en estos criterios, se utilizan para excluir otras patologías, sin existir en los criterios diagnósticos de 1984 ningún biomarcador de apoyo de la propia EA. Estos criterios diagnósticos han sido de gran utilidad en las últimas décadas, si bien el avance en el conocimiento de la enfermedad ha evidenciado varias limitaciones en su aplicación. Una de ellas es su relativa baja sensibilidad y especificidad, hecho reflejado en múltiples trabajos de correlación clínico-patológica con sensibilidades en torno al 80% y especificidades alrededor del 70% (Knopman et al., 2001; Beach et al., 2012). Las presentaciones atípicas de la enfermedad son una de las principales causas de la baja sensibilidad. Si bien en la inmensa mayoría de los casos el primer síntoma es un déficit de memoria episódica, en un porcentaje relevante de casos (5-10% en la EAT y hasta un tercio en la EAP) el primer síntoma es un trastorno del lenguaje, problemas visuoespaciales o visuoperceptivos o alteraciones de conducta y/o ejecutivas, lo que provoca que estos sujetos reciban con frecuencia diagnósticos diferentes al de EA (Warren et al., 2012). El hecho que otras enfermedades neurodegenerativas como la demencia frontotemporal o causas no-degenerativas, como las lesiones vasculares o algunas enfermedades psiquiátricas, puedan causar un cuadro clínicamente similar al causado por la EA disminuiría la especificidad de unos criterios diagnósticos puramente clínicos.

Otra limitación de los criterios clínicos NINCDS-ADRDA de 1984 es que únicamente permiten un diagnóstico de EA en una fase tardía de la enfermedad, ya que la presencia de demencia es un requisito ineludible para el diagnóstico. Múltiples estudios en las dos últimas décadas han demostrado que las primeras alteraciones biológicas a nivel cerebral están presentes décadas antes del desarrollo de demencia (Braak et al., 1991; Braak et al., 1997; Price et al., 1999). Todo ello ha llevado a que, en la última década, el concepto de EA haya cambiado sustancialmente. Actualmente, la corriente científica predominante considera que, de forma similar a la mayoría de enfermedades crónicas del ser humano, la EA tiene una fase a(pre)sintomática muy larga (que puede llegar hasta 20-30 años), durante la cual el proceso fisiopatológico de la enfermedad está activo, causando un acúmulo progresivo de patología cerebral y la consiguiente muerte neuronal, contrabalanceado inicialmente por mecanismos

compensatorios (Jack et al., 2010). Esta fase, por consenso, se ha denominado EA preclínica (Sperling et al., 2011). Cuando los mecanismos de compensación cognitiva se hacen insuficientes aparece la sintomatología de la enfermedad, que, en la mayoría de los casos, es un trastorno de la memoria episódica. En este nuevo paradigma, la demencia es solo la fase final de un largo *continuum* clínico-biológico que ha comenzado décadas antes.

En este *continuum* de la enfermedad, existe una fase intermedia entre la fase asintomática de la EA y su fase de demencia en la que los pacientes presentan una alteración cognitiva significativa pero sin cumplir criterios de demencia, por lo que se podrían definir clínicamente como pacientes con deterioro cognitivo leve (DCL). Este concepto se estableció para designar a sujetos con alto riesgo para desarrollar demencia, que presentaban en el momento de la evaluación una alteración cognitiva evidente tanto para ellos mismos como para el explorador pero sin afectación relevante de las ADL y sin criterios de demencia (Petersen et al., 1999). La principal limitación de este concepto es que el DCL es un síndrome clínico muy heterogéneo, con etiologías, sintomatología y evolución muy diferentes. La variante amnésica del DCL (DCLa) es la que tiene una mayor probabilidad de evolucionar a una EA durante el seguimiento con tasas de progresión a demencia de 10-15% al año, si bien entre 10-30% de los sujetos con DCLa no muestran en el estudio neuropatológico las lesiones típicas de EA (Jicha et al., 2006; Markesbery 2010). Por todo ello y con el objetivo de definir más específicamente el subgrupo de sujetos con DCLa que van a evolucionar a una demencia tipo EA en los últimos años se han realizado diferentes propuestas. En 2007, Dubois y cols proponen el término de "EA prodrómica" para aquellos pacientes que junto a la alteración de la memoria episódica presenten una alteración de alguno de los biomarcadores considerados específicos de la EA (atrofia medial temporal en RM, perfil típico en LCR, patrón de hipometabolismo característico o la presencia de una mutación causante de EA) en ausencia de demencia. El uso de marcadores bioquímicos nos permite realizar un diagnóstico más específico, al ser apoyado por una prueba biológica que marca los cambios patológicos subyacentes, y más precoz, al permitir el diagnóstico de EA en el momento de la objetivación de los primeros síntomas sin tener que esperar a la progresión clínica hasta la fase de demencia. La aceptación de estos criterios por la comunidad científica y las principales agencias reguladoras internacionales está permitiendo ya testar el efecto de potenciales fármacos modificadores del curso de la enfermedad en una fase predemencia. Sin embargo, aún en ausencia de un tratamiento probado eficaz en este momento para esta fase de la EA, un diagnóstico más precoz tiene diferentes ventajas prácticas. Así, podemos realizar un diagnóstico y un pronóstico más certero en un individuo

sintomático pero aún competente que podría tomar las decisiones pertinentes acerca de su futuro.

Posteriormente a la publicación de los criterios previamente mencionados, en 2011, y con la misma idea de poder identificar aquellos sujetos con DCL que podrían evolucionar a demencia tipo EA, el Instituto americano del envejecimiento en conjunción a la Asociación americana de Alzheimer revisaron los criterios de DCL, introduciendo varios niveles de probabilidad de que la causa subyacente de los síntomas fuera la patología tipo EA, en base a la alteración de uno o varios de los biomarcadores específicos de EA (Albert et al., 2011). El síntoma cardinal en la mayoría de los casos de EA es una afectación de la memoria episódica, reflejo de la afectación de la región temporal medial por depósitos neurofibrilares de proteína tau. Por ello, tanto los criterios iniciales de 1984 como su revisión en 2011 (McKhan et al., 1984; McKhan et al., 2011), inciden en la importancia de una anamnesis y exploración clínica cuidadosas para objetivar un síndrome amnésico del tipo hipocampal. Las pruebas neuropsicológicas más utilizados en la valoración cognitiva de estos sujetos han sido los de aprendizaje de nueva información con valoración de la capacidad de recuerdo diferido, mostrando una capacidad de diferenciar sujetos con una alteración de la memoria causada por EA de sujetos control con sensibilidades y especificidades superiores al 90% (Welsh et al., 1991). Además, las pruebas de recuerdo diferido libre y facilitado con pistas tipo Free and Cued Selective Reminding Test – FCSRT (Grober et al., 1988) son capaces de predecir que sujetos con DCL van progresar a demencia (Grober et al., 2000; Tierney et al., 2005; Bäckman et al., 2005; Sarazin et al., 2007; Wagner et al., 2012), evidenciando la necesidad de una buena exploración neuropsicológica, con especial énfasis en la valoración de la memoria episódica, en la evaluación de los pacientes con deterioro cognitivo.

Sin embargo, la sintomatología inicial de la EAP a menudo es diferente de la característica afectación de la memoria episódica de la mayoría de casos de EAT, hecho que conlleva una mayor dificultad en el diagnóstico de probabilidad basado únicamente en criterios clínicos en un porcentaje relevante de casos. El porcentaje de presentaciones atípicas de la EAP varía según las cohortes analizadas con cifras tan dispares como 20 o 60%, lo que sugiere que estas pueden estar sesgadas por diferentes variables, como podrían ser el tipo de centro donde se realiza el estudio (sesgo de remisión a centros terciarios) o si la cohorte es puramente clínica o clínico-patológica etc. (Koedam et al., 2010; Mendez et al. 2012). Estas presentaciones clínicas atípicas se han clasificado en tres grandes categorías: variante frontal, caracterizada por síntomas disejecutivos y conductuales prominentes (Alladi et al, 2007;

Taylor et al., 2008), variante del lenguaje, denominada afasia logopénica y caracterizada por una disminución de la velocidad de producción en el lenguaje espontáneo con pausas frecuentes para encontrar la palabra y alteración de la repetición de frases (Gorno-Tempini et al., 2008; Rabinovici et al., 2008) y variante posterior, en las cuales se incluirían los síndromes de la atrofia cortical posterior y el síndrome corticobasal (Benson et al., 1988; Ross et al., 1996; Aharon-Peretz et al., 1999; Migliaccio et al., 2009; Ling et al., 2010). Una manifestación clínica inicial diferente al síndrome amnésico hipocámpico hace más probable recibir un diagnóstico en vida diferente al de EA (Alladi et al., 2007; Ling et al., 2010; Snowden et al., 2011). Los pacientes con EAP, además, tienden a tener una disfunción cognitiva más generalizada desde el inicio de la sintomatología con mayores déficits en lenguaje, atención y funciones visuoespaciales y un deterioro cognitivo y funcional más rápido respecto a la EAT sugiriendo una mayor agresividad biológica de las formas de inicio precoz (Jacobs et al., 1994; Koss et al., 1996; Snowden et al., 2007; Smits et al., 2012).

El mejor conocimiento en los últimos años de algunas de estas presentaciones atípicas de la EA ha permitido que la revisión de los criterios diagnósticos de consenso de la demencia causada por la EA reconozca la existencia de presentaciones no-amnésicas de la EA (presentaciones de lenguaje, visuoespaciales y disejecutivas) (McKhan et al., 2011). Cabe pensar que es precisamente en esta categoría clínica donde el uso de marcadores biológicos podría tener una mayor relevancia en el diagnóstico ya que la sintomatología no-amnésica de estos sujetos se puede confundir frecuentemente con síntomas de la demencia frontotemporal o de enfermedades no-degenerativas, si bien los trabajos que ratifiquen esta hipótesis aún escasean.

En resumen, actualmente hay claras evidencias que en la EA existe un período preclínico, que puede durar años, seguido de un período prodrómico en cual aparecen los primeros síntomas. La correcta identificación de este período prodrómico permitiría adelantar el diagnóstico de la enfermedad antes de llegar a una fase de demencia con el apoyo de unos marcadores biológicos que aumenten la certeza de que la base biológica de la sintomatología es la EA. Este hecho es especialmente relevante cuando el inicio del deterioro cognitivo se produce en edades en las que las demencias neurodegenerativas son infrecuentes, dado que en general, la mayoría de los métodos diagnósticos utilizados han sido validados en población con EAT, y que las presentaciones clínicas de debut en este grupo de edad, como hemos comentado, son frecuentemente diferentes de las formas clásicamente descritas, lo que conjuntamente podría conllevar un mayor retraso diagnóstico y un mayor porcentaje de

error diagnóstico en la EAP con las repercusiones emocionales, socio-laborales y jurídicas que esto conllevaría. Así, conceptualmente, serían los sujetos con EAP los que potencialmente más se podrían beneficiar de la aplicación de los nuevos marcadores biológicos para el diagnóstico clínico.

2. Genética e hipótesis fisiopatológica de la enfermedad de Alzheimer: la cascada amiloide

La prevalencia de la EA causada por una mutación en un gen determinante es inferior al 1% del total de casos, si bien este porcentaje es significativamente superior en las formas de inicio precoz. Así, aproximadamente el 10% de los casos de EAP podrían ser causadas por mutaciones en uno de los 3 genes implicados en la EA monogénica: el gen de la proteína precursora de la amiloide (*APP*) y los genes de la presenilina 1 y 2 (*PSEN1* y *PSEN2*) (Lladó et al., 2006). Hasta la fecha se han descrito 33 mutaciones diferentes en *APP* (incluyendo mutaciones puntuales y duplicaciones), 185 en *PSEN1* y 13 en *PSEN2* (Alzheimer Disease. & Frontotemporal Dementia Mutation Database, http://www.molgen.vib-ua.be/ADMutations).

Los casos genéticos representan un modelo excepcional de enfermedad ya que son sujetos que tienen un desarrollo psicomotor normal durante las primeras décadas de vida. Los primeros síntomas suelen aparecer entre la tercera y la quinta década con una penetrancia prácticamente completa a los 65 años y una edad de inicio de la sintomatología que suele estar relativamente conservada dentro de una misma familia. Con cierta frecuencia en la literatura se han descrito variaciones fenotípicas como son la presencia de importante sintomatología motora como paraparesia (Dumanchin et al., 2006; Gómez-Tortosa et al., 2010) o la presencia de hematomas cerebrales en algunas mutaciones de *PSEN1* (Sánchez del Valle et al., 2007). Estudios en cohortes de portadores de diferentes mutaciones en *PSEN1* han demostrado que la enfermedad en estos sujetos puede tener una fase prodrómica muy larga, al objetivar déficits en la exploración neuropsicológica unos diez años antes de llegar a la fase de demencia (Acosta-Baena et al., 2011; Bateman et al., 2012).

Los tres genes causantes de EA monogénica (*PSEN1*, *PSEN2*, *APP*) están implicados en la producción y el procesamiento de la proteína amiloide: el gen *APP* sintetiza la proteína precursora del amiloide. Esta proteína se puede procesar a través de dos vías: una no-

amiloidogénica (mediante la acción de α y después γ-secretasas produciendo residuos pequeños e hidrosolubles) y otra amiloidogénica por la acción de la β y γ-secretasas, que acabará produciendo a nivel celular los diferentes monómeros de Aβ, principalmente de las isoformas de cadena larga (42 aminoácidos) que tienen más tendencia a oligomerizarse y posteriormente a formar amiloide fibrilar insoluble (Scheuner et al., 1996; Bentahir et al., 2006). Tanto el gen de la *PSEN1* como el *PSEN2* forman parte del sitio catalítico activo de la γ-secretasa y las mutaciones es estos genes producen alteraciones del funcionamiento normal del gen con un aumento neto de la producción de las isoformas amiloidogénicas.

La hipótesis más aceptada actualmente sobre la fisiopatología de la EA es la hipótesis de la cascada amiloide (Hardy et al., 1992). Este modelo se basa fundamentalmente en la información derivada de los hallazgos genéticos o modelos animales portadores de mutaciones causantes de la enfermedad. Según esta hipótesis, el acontecimiento inicial en la fisiopatología de la enfermedad sería el acúmulo de isoformas de β-amiloide (Figura 1). Este acúmulo se produciría a consecuencia de un desequilibrio entre la producción y el aclaramiento cerebral del β-amiloide. Así, el mecanismo fisiopatológico principal en los casos genéticos sería el aumento de producción de β-amiloide. Por el contrario, si bien probablemente estemos delante de un fenómeno complejo y multifactorial, el principal mecanismo patogénico en la enfermedad esporádica sería la disminución del aclaramiento del β-amiloide (Mawuenyega et al., 2010; Huang et al., 2012). Posteriormente, el acúmulo de β-amiloide desencadenaría una cascada de acontecimientos que conducirían a la fosforilación anómala de la proteína tau, formación de ovillos neurofibrilares, respuesta inflamatoria, disfunción sináptica y muerte neuronal con la consiguiente aparición de los síntomas.

El factor de riesgo genético más importante relacionado con la EA esporádica es el genotipo del gen de la apolipoproteína E (*APOE*). *APOE* es un gen que regula el metabolismo lipídico, mediando en el transporte transmembrana de lipoproteínas. En la periferia, la proteína apoE es producida en el hígado y macrófagos mientras que la principal fuente de apoE intracerebral son los astrocitos (Bu, 2009).

El gen *APOE* existe en el ser humano en forma de tres alelos ε2, ε3 and ε4, que tienen una frecuencia global de aproximadamente 1-10%, 60-80% y 5-20% respectivamente. Se ha observado que la frecuencia del alelo ε4 está incrementada de forma relevante en sujetos afectos de EA (aproximadamente el 50% tienen al menos un alelo ε4) (Corder et al., 1993).

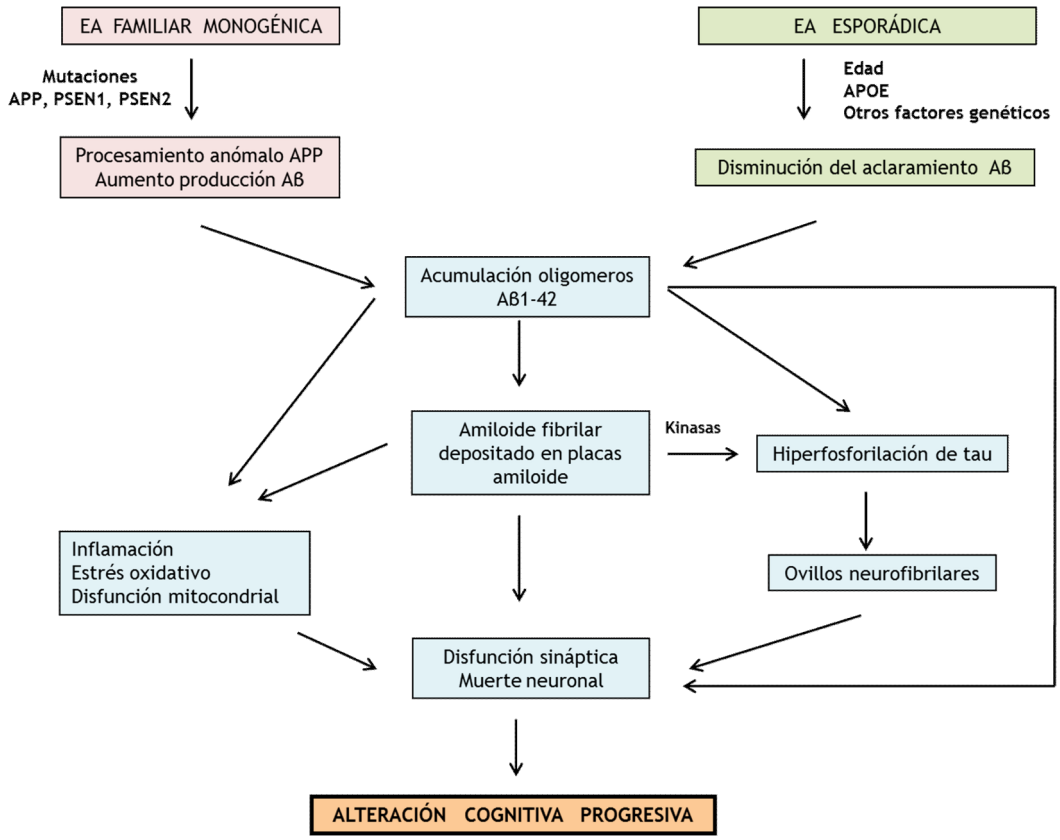

FIGURA 1 - La hipótesis de la cascada amiloide en la enfermedad de Alzheimer (adaptado de Blennow et al., 2010)

Asimismo, estudios poblacionales habían estimado que la presencia de un alelo ε4 incrementa el riesgo de EA por un factor de 3, mientras que los homocigotos ε4ε4 tienen aproximadamente 14 veces más riesgo de presentar la enfermedad que los no portadores (Farrer et al., 1997). Los mecanismo por los cuales el alelo ε4 aumenta el riesgo de EA son complejos y no completamente esclarecidos. Probablemente el efecto principal se produce mediante la interferencia en el metabolismo y aclaramiento del β-amiloide, aumentando la velocidad de depósito cerebral respecto a los alelos ε3 y ε2 (Holtzman et al., 2000; Vermuri et al., 2010), aunque seguramente en la aparición de la enfermedad están implicadas también vías independientes de β-amiloide como las involucradas en la plasticidad sináptica, la disminución del metabolismo de la glucosa, la regulación de la respuesta inflamatoria, la disminución de la neurogénesis y/o disfunción vascular (Dumanis et al., 2009; Sheline et al., 2010; Chen et al., 2010; Filippini et al., 2011; Verghese et al., 2011), si bien se discute si estos factores inciden en la frecuencia de aparición de la enfermedad en sí o actúan

modificando la edad de inicio de la sintomatología. Los portadores del alelo ε4 muestran mayor hipometabolismo cerebral en FDG-PET, mayor atrofia cortical e hipocámpica y mayor depósito de β-amiloide en fases presintomáticas (Meyer et al., 1998; Reiman et al., 2004; Morris et al., 2010; Kantarci et al., 2012; Protas et al., 2013). Asimismo, se ha demostrado en portadores de ε4 una mayor velocidad de progresión de atrofia hipocámpica y de progresión clínica a demencia una vez aparecida la sintomatología (Schuff et al., 2009; Caselli et al., 2009; Lo et al., 2011). También hay trabajos que relacionan la presencia de un alelo ε4 con una presentación típica y su ausencia con una presentación atípica de la EA (van del Vlies et al., 2007) aunque el número de sujetos analizado es relativamente pequeño y con ausencia de confirmación neuropatológica.

Las nuevas técnicas disponibles en los últimos años, como el *Genome wide Association studies* (GWAS) y las técnicas de *microarrays* de expresión génica han permitido ampliar nuestro conocimiento sobre las bases genéticas de la EA. Así, mediante GWAS se han se han descrito otros factores genéticos, además del *APOE*, que contribuyen a explicar parte de la heredabilidad de la enfermedad en casos no portadores de *APOE* ε4 (Harold et al., 2009), como serían polimorfismos en *CLU, CR1, PICALM, SORL1* y otros, que parecen estar implicadas en aspectos tan diversos como el procesamiento de β-amiloide, la regulación de la respuesta inmune e inflamación, procesamiento lipídico, transporte transmembrana o la plasticidad sináptica. Las técnicas de los *microarrays* permiten estudiar de forma simultánea un conjunto de genes que se están expresando en un tipo celular concreto en una situación determinada. Resultados obtenidos en la última década mediante esta técnica han implicado en la fisiopatogenia de la EA vías metabólicas relacionadas con el funcionamiento sináptico, regulación de neurotransmisores, señalización intracelular vía calcio, metabolismo energético y el estrés oxidativo. Todos estos estudios se han enfocado en la enfermedad de inicio tardío, con muy pocos trabajos en EAP.

3. Marcadores biológicos de la enfermedad de Alzheimer

Un biomarcador se define como una característica biológica individual que se puede evaluar y medir de forma objetiva como indicador de un proceso biológico normal, patológico o de respuesta farmacológica a una intervención terapéutica específica (*Biomarkers Definitions Working Group,* 2001). En los últimos años los avances tecnológicos han permitido que alteraciones claves señaladas en la hipótesis de la cascada fisiopatológica de la EA se puedan

objetivar *in vivo* mediante marcadores específicos. Dependiendo del proceso fisiopatológico subyacente que evalúan estos marcadores se han clasificado en marcadores de amiloidosis y marcadores de neurodegeneración (Sperling et al., 2011). Los marcadores de amiloidosis están representados por el estudio de la isoforma 1-42 de la proteína β-amiloide (Aβ42) en el LCR y los estudios de PET con trazadores de β-amiloide y evidenciarían *in vivo* el depósito cerebral de β-amiloide mientras que los marcadores de neurodegeneración serían el reflejo de la disfunción sináptica y posterior muerte neuronal y están representados por los niveles de la proteína tau total (t-tau) y proteína tau hiperfosforilada (p-tau) en el LCR, los cambios estructurales objetivados por la RM y el estudio del metabolismo cerebral de glucosa mediante estudios PET.

Según se recogen en los nuevos criterios diagnósticos de consenso, la positividad de un marcador de amiloidosis y uno de daño neuronal aumentarían la evidencia de que la patología subyacente a un DCL o una demencia sea realmente una EA a un grado de alta probabilidad (Albert et al, 2011; McKhan et al., 2011).

3.1 Marcadores de amiloidosis

Líquido cefalorraquídeo

Dado que la patología de la EA está confinada en el cerebro, el LCR es un candidato idóneo para el estudio de marcadores bioquímicos que reflejen la patología de la enfermedad. El marcador de amiloidosis más utilizado para el diagnóstico de EA en LCR ha sido los niveles de Aβ42, que en pacientes con EA se encuentran disminuidos. La disminución del Aβ42 se correlaciona con el depósito parenquimatoso de β-amiloide en forma de placas amiloideas, habiendo una correlación clara entre la disminución del Aβ42 en el LCR, la captación aumentada en el PET de amiloide y la cantidad de placas amiloideas en el estudio neuropatológico (Strozyk et al., 2003; Buerger et al., 2006; Engelborghs et al., 2008; Shaw et al., 2009; Tapiola et al., 2009; Seppälä et al., 2012). Los niveles de Aβ42 se encuentran ya disminuidos en el momento de la aparición de los síntomas tanto en casos esporádicos como en portadores de mutaciones (Fagan et al., 2007; Fortea et al., 2011; Bateman et al., 2012).

Estudios PET con trazadores de β-amiloide

El PET con trazadores para depósitos de amiloide fibrilar, de los cuales el primero en utilizarse fue el *Pittsburg Compound B* marcado con ^{11}C - ^{11}C-PIB, muestra una captación aumentada del trazador en zonas frontales, temporoparietales y estriatales en la inmensa mayoría de sujetos con EA respecto a controles (Klunk et al., 2004). La técnica ha sido validada en estudios de correlación clínico-patológicos (Ikonomovic et al., 2008; Leinonen et al., 2008). Nuevos trazadores desarrollados recientemente validados en cohortes patológicas (Clarck et al., 2012) contribuirán probablemente en el futuro próximo a aumentar la accesibilidad de la prueba.

3.2 Marcadores de neurodegeneración

Líquido cefalorraquídeo

Los marcadores de neurodegeneración más validados en el LCR en la EA son los niveles de t-tau y p-tau. La proteína tau es un componente normal del citoesqueleto axonal y en su forma fosforilada es el principal componente de los ovillos neurofibrilares. En la EA, los niveles de t-tau y p-tau reflejarían el daño neuronal ya que se liberaría al espacio extracelular/LCR tras la muerte neuronal. Hay una correlación robusta entre el aumento de los niveles de t-tau y p-tau y la extensión de la pérdida neuronal y patología neurofibrilar en el estudio neuropatológico (Strozyk et al., 2003; Buerger et al., 2006; Engelborghs et al., 2008; Shaw et al., 2009; Josephs et al., 2008; Tapiola et al., 2009). La determinación de su isoforma fosforilada en LCR se correlaciona de forma más específica con los ovillos neurofibrilares y permitiría aumentar la especificidad del diagnóstico (Koopman et al., 2009).

Resonancia magnética estructural

Las técnicas de neuroimagen estructural han sido utilizadas para el diagnóstico clínico de la EA desde hace décadas para objetivar la atrofia cerebral secundaria a los cambios patológicos y para descartar diagnósticos alternativos. Técnicas como la volumetría basada en vóxeles han permitido el uso de las técnicas de neuroimagen en la investigación, evidenciado el papel de la atrofia hipocámpica en el diagnóstico de la EA y la correlación

directa que existe entre el volumen del hipocampo y el rendimiento cognitivo en el *continuum* envejecimiento normal-DCL-demencia tipo EA (Jack et al., 1997; Barnes et al., 2009). En sujetos con DCL la atrofia del hipocampo es un buen predictor de progresión clínica a demencia (Lo et al., 2011; Landau et al., 2012) y de patología tipo EA en el examen postmortem (Jack et al., 2002; Silbert et al., 2003).

Nuevas técnicas semiautomáticas puestas a punto en los últimos años, como la evaluación del grosor cortical, han permitido delinear un patrón de atrofia cortical característico de la EA que abarcaría zonas como la región temporal medial, temporoparietal lateral, precuneus o el cíngulo posterior y que permitiría discriminar con alta fiabilidad entre sujetos afectos y controles (Desikan et al., 2009). Este patrón cortical de atrofia es muy robusto y se ha podido identificar en sujetos en fase de DCL y que posteriormente progresan a una demencia tipo EA (Dickerson et al., 2009; Bakkour et al., 2009).

La mayoría de estos estudios se han llevado a cabo en cohortes de EAT. Estudios comparativos entre pacientes con EAP y EAT han descrito diferencias en el patrón de atrofia cortical. Así, los sujetos con EAP presentarían mayor atrofia de sustancia gris en el cíngulo posterior, precuneus, zonas frontales y temporoparietales de asociación mientras que los sujetos con EAT tendrían una atrofia más circunscrita a la región temporal (Ishii et al., 2005; Frisoni et al., 2007). La EAP tiene, en general, una progresión más rápida de la atrofia cerebral global y del grosor cortical así como de estructuras subcorticales (tálamos, núcleos lenticulares, amígdala) respecto a la EAT y un patrón más difuso de alteración de los tractos de sustancia blanca (Chan et al., 2003; Canu et al., 2010; Cho et al. 2013).

Estudio longitudinales en portadores de mutaciones han demostrado que estos sujetos presentan cambios estructurales objetivables y característicos de la enfermedad (atrofia hipocámpica, atrofia temporoparietal, cíngulo posterior y precuneus) hasta cinco años antes de la aparición del primer síntoma (Ridha et al., 2006; Bateman et al., 2012). Este patrón de atrofia progresa y se generaliza a medida que la enfermedad va evolucionando. Hasta ahora la información disponible en relación a las posibles diferencias en el patrón de atrofia cortical en RM entre sujetos con EAP esporádica y EAP genética es prácticamente inexistente.

PET con trazadores de glucosa

El PET de fluorodeoxiglucosa (^{18}F-FDG PET) muestra en pacientes con EA un patrón característico de hipometabolismo cerebral temporoparietal bilateral, en cingulado posterior y en precuneus, que progresa a medida que la enfermedad avanza (Alexander et al., 2002). La disminución del metabolismo regional cerebral se correlaciona con la progresión de los déficits cognitivos desde DCL hasta una fase de demencia (Drzezga et al., 2003; Engler et al., 2006). La técnica presenta buena sensibilidad y especificidad, en torno al 85%, aunque con importantes variaciones entre diferentes trabajos, para diferenciar entre sujetos con EA y sujetos sin enfermedad neurodegenerativa (Patwardhan et al., 2004) o sujetos con demencia frontotemporal (Foster et al., 2007; Panegyres et al., 2009). La presencia de hipometabolismo cortical se asocia a la progresión clínica de los síntomas en sujetos con deterioro cognitivo leve (Landau et al., 2012). Los sujetos con EAP parecen tener mayor hipometabolismo cortical ajustando por el nivel de deterioro cognitivo respecto a sujetos con EAT (Rabinovici et al., 2010).

3.3 Modelo integrado de alteración secuencial y relación entre diferentes biomarcadores en la Enfermedad de Alzheimer

La mayoría de los marcadores biológicos de la EA mencionados previamente están ya alterados en el momento de la aparición de los síntomas. Se ha postulado que la alteración cronológica de estos marcadores seguiría un orden predeterminado (Jack et al., 2010; Jack et al., 2013). Así, el acontecimiento inicial de la enfermedad sería el procesamiento anómalo del β-amiloide que acaba secuestrado en su forma fibrilar en las placas amiloideas ya en fases presintomáticas de la enfermedad. El depósito de β-amiloide llega prácticamente a un *plateau* probablemente antes del inicio de la sintomatología, hecho evidenciado tanto en cohortes con enfermedad esporádica como genética (Villemagne et al., 2013; Bateman et al., 2012). La siguiente alteración en aparecer sería la disfunción sináptica y la muerte neuronal objetivable mediante la elevación de los niveles de la proteína tau en LCR y/o en cambios metabólicos en el FDG-PET. Tardíamente, la pérdida neuronal se podría cuantificar a nivel de la RM estructural. Finalmente, todo ello conduciría a la aparición de los primeros síntomas clínicos, que posteriormente progresarían hasta la fase de demencia. Así, las alteraciones del funcionamiento diario secundarias a los déficits cognitivos que aparecen en la fase de demencia serían solo el acontecimiento final de toda una cadena de eventos que

posiblemente abarca décadas. Factores como la reserva cognitiva o la presencia de copatología cerebral pueden modular la expresión clínica y la progresión de los déficits cognitivos (Vermuri et al., 2011; Ewers et al., 2013). Este modelo teórico descrito ha sido parcialmente validado en diferentes cohortes clínicas tanto transversales como longitudinales de corta duración, tanto en enfermedad esporádica (Lo et al., 2011; Jack et al., 2011; Forster et al., 2012; Landau et al., 2012; Villemagne et al., 2013) como en portadores de mutaciones (Bateman et al., 2012; Fleisher et al., 2012). Habría que mencionar no obstante que este modelo no pretende ser exhaustivo sino meramente un marco para centrar los nuevos trabajos e interpretar los resultados de los mismos.

Los biomarcadores de la EA tienen correlatos diferentes a lo largo del *continuum* biológico de la enfermedad. En la fase preclínica es la amiloidosis cerebral la que mejor se correlaciones con los hallazgos estructurales o cognitivos. Pero, una vez han aparecido los síntomas se ha reportado poca correlación entre los marcadores de amiloidosis y la atrofia cerebral o la progresión clínica (Fagan et al., 2007; Fagan et al., 2009; Chételat et al., 2010; Villemagne et al., 2013). En la fase de DCL y demencia son los niveles de t-tau y p-tau los que correlacionan de forma directa con la estructura cerebral y la cognición. Los sujetos con DCL en los que se evidencia un perfil típico de EA son los que, durante el seguimiento, tienen más probabilidad de progresar a una demencia tipo EA (Hansson et al., 2006; Mattsson et al., 2009). Además, niveles altos de t-tau y p-tau predicen una progresión clínica más rápida a demencia en sujetos con DCL (Buchhave et al., 2012), y una mayor progresión de la sintomatología una vez que se ha llegado a la fase de demencia (Wallin et al., 2008; van der Vlies et al., 2009). La mayoría de los estudios que han validado el uso de biomarcadores en LCR se han realizado en EAT, existiendo muy pocos estudios que los evalúen en población menor de 65 años. Schoonenboom y colaboradores (Schoonenboom et al., 2004) confirman la presencia del perfil típico de LCR en sujetos con EAP con sensibilidades y especificidad por encima del 80% para diferenciarlos de sujetos con demencia frontotemporal o controles. Los trabajos más consistente que comparan sujetos con EAP y EAT no encuentran diferencias relevantes entre los niveles de los tres biomarcadores cardinales en LCR, si bien en los respectivos grupos de sujetos control ven un aumento de la probabilidad de tener un marcador alterado a mayor edad, sugiriendo una mayor especificidad del diagnóstico bioquímico en la enfermedad de inicio precoz (Sjögren et al., 2000; Bouwman et al., 2009; Antonell et al., 2011). Para intentar disminuir este solapamiento entre sujetos control y pacientes con EA se ha propuesto la utilización de

índices compuestos de LCR que combinen los niveles de Aβ42 con los de tau, de ellos el más utilizado ha sido el ratio entre Aβ42 y p-tau (Fagan et al. 2007, Welge et al. 2009).

De los diferentes biomarcadores comentados hasta ahora en la presente tesis doctoral se evaluarán especialmente aquellos relacionados con el LCR y los marcadores de neuroimagen estructural.

4. Neuropatología de la enfermedad de Alzheimer

Los criterios clínicos de EA establecen un papel primordial de la anatomía patológica en el diagnóstico definitivo la EA, puesto que el estudio neuropatológico es imprescindible para poder establecerlo (McKhan et al., 1984; McKhan et al., 2011). A nivel neuropatológico, la EA se caracteriza por la presencia de placas amiloideas extracelulares (compuestas por β-amiloide fibrilar, predominantemente la isoforma Aβ42), ovillos neurofibrilares intracelulares (compuestas por la proteína tau hiperfosforilada) y una combinación de las dos patologías, las placas neuríticas (con un centro de β-amiloide rodeado por neuritas distróficas con depósitos de tau fosforilada y reacción inflamatoria). Tanto la proteína amiloide como la proteína tau son componentes normales de las neuronas, pero, en el caso de EA, adoptan conformaciones fibrilares que las hacen insolubles, lo que provoca que acaben acumulándose en el parénquima cerebral desencadenando una cascada de eventos que desembocarán en la muerte neuronal y la aparición de un deterioro cognitivo progresivo.

Tanto el depósito de β-amiloide como la fosforilación de tau parecen tener una progresión anatómica estereotipada en el tiempo (Braak et al., 1997; Alafuzoff et al., 2008; Alafuzoff et al., 2009). Cuando el sujeto presenta los primeros síntomas de la enfermedad suele tener ya marcados cambios a nivel neuropatológico. Para el diagnóstico patológico de EA los criterios vigentes recomiendan una evaluación sistemática de varias áreas cerebrales valorando la existencia y el grado de extensión de las lesiones típicas de la enfermedad (depósitos de β-amiloide, ovillos neurofibrilares y placas neuríticas), valoración de la afectación vascular por depósitos de β-amiloide, así como una evaluación de la posible contribución a la sintomatología clínica de lesiones concomitantes (como las lesiones vasculares u otros depósitos proteicos diferentes a la EA). En base a ello se dictamina el grado de probabilidad de que las manifestaciones clínicas que había presentado el sujeto en vida fueran causadas por una EA (Montine et al., 2012).

Los cambios patológicos son cualitativamente similares en todos los sujetos independientemente de la edad de inicio. Sin embargo, hay trabajos que sugieren que los pacientes con EAP pueden tener mayor carga amiloide y neurofibrilar y mayor pérdida sináptica y neuronal respecto a la enfermedad de inicio tardío (Bigio et al., 2002; Marshall et al., 2007) sugiriendo una mayor agresividad de las formas de inicio precoz, si bien estos datos tienen que ser interpretados con cautela ya que podría tratarse simplemente de un sesgo debido a que los pacientes de menor edad viven más tiempo con la enfermedad al tener menos comorbilidad.

Asimismo, los hallazgos neuropatológicos en los casos genéticos también son cualitativamente similares a los observados en casos esporádicos. No obstante, se han descrito peculiaridades tanto a nivel de morfología de las lesiones como a nivel cuantitativo. Los depósitos de β-amiloide en los casos genéticos adoptan a veces una conformación peculiar en forma de placas algodonosas, de mayor tamaño que las placas seniles y con poca reacción inflamatoria y neuritas distróficas perilesionales (Crook et al., 1998; Brooks et al., 2003; Dumanchin et al., 2006) si bien estos hallazgos no son exclusivos de esta etiología al haberse descrito también de forma infrecuente en la EA esporádica (Yokota et al., 2003).

Los sujetos portadores de mutaciones tienen mayor carga total de β-amiloide a nivel cerebral y específicamente de Aβ42 a nivel de placas respecto a los casos esporádicos, así como mayor atrofia hipocámpica y cortical y mayor o igual carga neurofibrilar, aunque estos hallazgos podrían variar dependiendo de la localización de la mutación (Iwatsubo et al., 1994; Gomez-Isla et al., 1999; Ishii et al., 2001; Mann et al., 2001; Sudo et al., 2005; Gregory et al., 2006; Shepherd et al., 2007). A nivel de lesión vascular, si bien es un fenómeno general en todos los casos de EA independientemente de la etiología, ciertas mutaciones se asocian a extensos depósitos de Aβ40 en la pared vascular, produciendo una marcada angiopatía amiloidea con eventos hemorrágicos de repetición (Natté et al., 2001; Sánchez-Valle et al., 2007). Sin embargo, la mayoría de estas comparaciones se han realizado entre casos genéticos y EAT esporádica y prácticamente no existen trabajos que comparen las posibles diferencias entre casos genéticos y EAP.

En este contexto, el objetivo general de la presente tesis doctoral es intentar caracterizar mejor la EA de inicio precoz esporádica mediante la evaluación de las presentaciones clínicas de inicio, el análisis del perfil de marcadores en el LCR, el estudio de la estructura

cerebral en RM y el patrón de expresión génica a nivel cerebral y comparar los hallazgos con aquellos obtenidos en pacientes con EA genéticamente determinada. Todo ello debería contribuir al aumento del conocimiento clínico y biológico de la EA de inicio precoz esporádica y a poder diagnosticar mejor y más precozmente los pacientes afectos, facilitando la aplicación futura de tratamientos específicos modificadores de la evolución de la enfermedad.

III.

HIPÓTESIS

HIPÓTESIS

HIPÓTESIS

1. La enfermedad de Alzheimer de inicio precoz esporádica se manifestaría clínicamente con frecuencia con sintomatología atípica, es decir, sin una afectación predominante de la memoria episódica como primer síntoma de la enfermedad. El genotipo de *APOE* y la presencia de copatología podría modificar la presentación clínica de pacientes con enfermedad de Alzheimer de inicio precoz esporádica.

2. La enfermedad de Alzheimer de inicio precoz esporádica presentaría un patrón típico de alteración de los marcadores bioquímicos en LCR y de alteración morfométrica en la RM craneal. Los pacientes con enfermedad de Alzheimer de inicio precoz esporádica podrían presentar diferencias cuantitativas en marcadores de amiloidosis o de neurodegeneración en LCR y/o neuroimagen estructural respecto a sujetos con enfermedad de Alzheimer de inicio precoz genéticamente determinada.

3. Los sujetos con enfermedad de Alzheimer de inicio precoz esporádica mostrarían un perfil de expresión génica cerebral alterado en múltiples vías biológicas, que podría ser diferente al perfil expresado por pacientes con enfermedad de Alzheimer de inicio precoz genéticamente determinada.

4. Los niveles de Aβ42, tau total y tau hiperfosforilada en LCR podrían tener una alta sensibilidad y especificidad para el diagnóstico precoz de enfermedad de Alzheimer en pacientes con deterioro cognitivo de inicio presenil permitiendo aumentar la probabilidad de que el proceso fisiopatológico subyacente al deterioro cognitivo sea una EA, ayudar al diagnóstico diferencial con otras enfermedades neurodegenerativas y pronosticar qué pacientes con DCL evolucionarán a demencia.

5. En pacientes con enfermedad de Alzheimer de inicio precoz y antecedentes familiares de demencia de inicio precoz estaría indicado realizar un estudio de genes determinantes por la posibilidad de identificar mutaciones patogénicas.

IV.

OBJETIVOS

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1. Estudiar la frecuencia de las diferentes presentaciones clínicas en una cohorte de sujetos con enfermedad de Alzheimer de inicio precoz esporádica con confirmación neuropatológica de la enfermedad. Evaluar el papel del genotipo de *APOE* y de las lesiones neuropatológicas concomitantes en la presentación clínica de la enfermedad.

2. Cuantificar las alteraciones en marcadores bioquímicos en LCR y la pérdida de grosor cortical en pacientes con enfermedad de Alzheimer de inicio precoz esporádica y compararlos con pacientes con enfermedad de Alzheimer de inicio precoz genéticamente determinada por la presencia de mutaciones en el gen *PSEN1*.

3. Estudiar el patrón de expresión génica cerebral a nivel del cingulado posterior en sujetos con enfermedad de Alzheimer precoz esporádica y compararlo con controles y sujetos con enfermedad de Alzheimer precoz causada por mutaciones en el gen *PSEN1*.

4. Evaluar la sensibilidad y especificidad de los niveles de Aβ42, tau total y tau hiperfosforilada en LCR para el diagnóstico diferencial de la enfermedad de Alzheimer en una cohorte de pacientes con deterioro cognitivo de inicio precoz. Analizar el cambio en el grado de probabilidad que la causa subyacente de los síntomas sea un proceso fisiopatológico de EA en pacientes con demencia de inicio precoz tras la aplicación de los biomarcadores de LCR según la nueva propuesta de criterios diagnósticos. Estudiar la capacidad de los biomarcadores de LCR de predecir la progresión a demencia tipo EA en sujetos con deterioro cognitivo leve de inicio precoz.

5. Describir una nueva mutación en el gen de *PSEN1* (L235R) en una familia con enfermedad de Alzheimer de inicio precoz.

V.

MÉTODOS

MÉTODOS

Los aspectos metodológicos se explican de forma detallada en los respectivos apartados de métodos de cada uno de los trabajos que componen esta tesis doctoral. Aquí solamente se resumen los criterios utilizados para la selección de las dos cohortes analizadas y se enumeran brevemente las técnicas utilizadas. Finalmente hay un breve apartado de aspectos éticos relacionados con la investigación clínica.

Diseño

Se han analizado dos cohortes de sujetos, la primera de tipo retrospectivo y la segunda de tipo prospectivo. Todos los análisis realizados son transversales. Todos los sujetos con diagnóstico clínico provienen de la Unidad de Alzheimer y otros trastornos cognitivos del Hospital Clínic (UATC) de Barcelona y los casos con confirmación neuropatológica del Biobanco-Banco de tejidos neurológicos (BTN) del Instituto de investigaciones biomédicas August Pi i Sunyer, a excepción de algunos casos controles sin evidencia anatomopatológica de enfermedad neurodegenerativa facilitados por el Biobanco del Hospital Universitario de Bellvitge.

Sujetos

Cohorte de pacientes con enfermedad de Alzheimer de inicio precoz con confirmación neuropatológica.

De la bases de datos del BTN se seleccionaron los casos disponibles con el diagnóstico neuropatológico de EA y una edad de inicio de los síntomas por debajo de los 60 años. Se eligió una edad de inicio menor a los 60 años con el objetivo de evitar un posible sesgo en la recogida retrospectiva de datos y asegurar únicamente la selección de casos con EAP. Los donantes procedían de diversos centros de Cataluña. El diagnóstico patológico de EA había sido realizado por neuropatólogos expertos acorde a los criterios diagnósticos vigentes (Braak et al. 1991, Braak et al. 1997, Alafuzoff et al. 2008, Alafuzoff et al. 2009, Montine et al. 2012). Esta cohorte ha sido utilizada en dos de los trabajos de esta tesis (trabajos 1 y 3).

Cohorte de pacientes con diagnóstico clínico de enfermedad de Alzheimer y otros tipos de deterioro cognitivo de inicio precoz.

Los sujetos fueron seleccionados entre los pacientes atendidos en una consulta específica enfocada a pacientes con deterioro cognitivo de inicio precoz de la UATC del servicio de neurología del Hospital Clínic. Para el trabajo 2 se reclutaron pacientes con EA precoz esporádica y portadores de mutaciones determinantes de la enfermedad, en fases sintomáticas, bien en fase de deterioro cognitivo leve o en fase de demencia (Global deterioration scale, GDS ≥3) (Reisberg et al. 1982) diagnosticados acorde a los criterios clínicos vigentes en el momento de la inclusión. En el trabajo 4 se reclutaron de forma secuencial y prospectiva sujetos que acudieron a la UATC para el estudio de deterioro cognitivo presenil. Se seleccionaron para establecer comparaciones controles cognitivamente sanos.

Recogida de datos y clasificación clínica

Cohorte EAP con confirmación neuropatológica

Se revisaron de forma retrospectiva los datos clínicos y patológicos de los sujetos seleccionados y se clasificaron en EAP esporádica o genética según la ausencia/presencia de mutaciones determinante de EA. Se clasificaron las presentaciones clínicas como típicas (cuando el primer síntoma era una alteración de la memoria episódica) o atípicas (cuando el primer síntoma era una alteración del lenguaje, visuoespacial/visuoperceptivo o conductual).

Cohorte con diagnóstico clínico EAP y otros tipos de deterioro cognitivo de inicio precoz

A todos los sujetos se les realizó una evaluación clínica y neuropsicológica completas, una RM estructural y una punción lumbar para la cuantificación de biomarcadores de EA en LCR (Aβ42, tau-total y tau-fosforilada). Los procedimientos de selección y estudio se detallan en los apartados de métodos de los respectivos trabajos.

Intervenciones

Estudios neuropsicológicos

En la cohorte clínica se ha administrado una batería completa de pruebas neuropsicológicas para establecer la presencia y el grado de los déficits cognitivos y que comprendía como mínimo dos pruebas para evaluar cada uno de los siguientes dominios cognitivos: memoria episódica, lenguaje, praxias, percepción visual, funciones ejecutivas frontales y pruebas atencionales. Se han utilizado los datos normativos disponibles previamente validados en nuestra población.

Estudio de biomarcadores en LCR

Se ha realizado el estudio de niveles de Aβ1-42, tau total y tau fosforilada en LCR por técnicas de ELISA con kits comerciales disponibles para tal fin de la compañía Innogenetics® (INNOTEST® ß-AMYLOID (1-42), INNOTEST® hTauAg e INNOTEST® PHOSPHO-TAU (181P)) siguiendo el protocolo del fabricante. Todas las determinaciones de LCR y parte de los estudios de búsqueda de mutaciones se han llevado a cabo en el laboratorio de enfermedades neurodegenerativas del IDIBAPS situado en el Centro de Investigación Biomédica CELLEX.

Estudios de neuroimagen

Todas las imágenes se adquirieron en un único aparato de RM 3.0 Tesla Siemens Magnetom Trio (Erlangen, Germany) de la Plataforma de imagen médica del IDIBAPS. El análisis de los datos se ha realizado en el Laboratorio de Neuroimagen del Departamento de Psiquiatría y Psicobiología Clínica de la Facultad de Medicina de la Universidad de Barcelona. El protocolo de adquisición de imágenes incluyó secuencias estructurales de alta resolución T1WI (3D MPRAGE) con un tamaño de voxel de 0,9 x 0,9 x 0.9 y secuencias ponderadas en T2/ FLAIR tridimensional para descartar lesiones isquémicas crónicas. Un neuroradiólogo experto valoró todas las secuencias y puntuó las imágenes mediante la escala de Fazekas (Fazekas et al. 1987). Para el análisis de imagen se han utilizado los métodos de grosor cortical (programa *FreeSurfer image analysis suite* http://surfer.nmr.mgh.harvard.edu/) y

análisis de morfometría basada en voxel (programa FSL-VBM (http://www.fmrib.ox.ac.uk/fsl/,Version 4.1.5).

Estudios genéticos

En todos los sujetos se realizó el genotipado del gen *APOE* mediante amplificación por PCR y digestión enzimática posterior según protocolos estándar. Siguiendo el protocolo diagnóstico del Programa de información y consejo genético para demencias familiares (PICOGEN) del Hospital Clínic, se realizó estudio de genes determinantes de EA genética mediante la secuenciación directa de los genes de la *PSEN1, PSEN2* y *APP*, así como la búsqueda de duplicaciones de *APP* en los pacientes con EAP e historia familiar de enfermedad de inicio precoz. Asimismo, en casos seleccionados, tal como se detalla en el trabajo número 4, se realizó la secuenciación de los genes *MAPT* o *GRN* así como el estudio de la presencia de expansión en el hexanucleótido situado en *C9ORF72*.

Análisis estadístico de los resultados

El análisis estadístico se ha llevado a cabo con el paquete SPSS para Windows (IBM Corporation, versión 19.0). Las variables cuantitativas se han analizado con el t de Student o U de Mann-Whitney según la distribución de los datos. En el caso de comparar varios grupos se ha realizado un análisis de la varianza (ANOVA) o un test de Kruskal-Wallis en función de la normalidad de distribución de los datos. Las variables categóricas se han comparado a través de test de χ^2 o el test exacto de Fisher.

Aspectos éticos

Los estudios que constituyen esta tesis doctoral han sido aprobados por el Comité ético de investigación clínica del Hospital Clínic. Todos los participantes en los estudios clínicos han firmado un consentimiento informado previamente valorado por el Comité ético del centro. Los estudios genéticos a pacientes se han realizado en el marco del programa PICOGEN del Hospital Clínic. Los trabajos que incluyen a sujetos procedentes del BTN han sido autorizados por el Comité científico del BTN. Todos los donantes de tejido neurológico o sus representantes legales habían firmado un consentimiento previo a la donación autorizando la utilización con fines científicos de sus tejidos neurológicos.

VI.

RESULTADOS

RESULTADOS

Trabajo número 1

Clinical features and APOE genotype of pathologically proven early-onset Alzheimer disease

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RESULTADOS

Clinical features and *APOE* genotype of pathologically proven early-onset Alzheimer disease

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ABSTRACT

Objectives: Early-onset Alzheimer disease (EOAD) diagnosis often represents a challenge because of the high frequency of atypical presentations. Our aim was to describe the clinical features, *APOE* genotype, and its pathologic correlations of neuropathologic confirmed EOAD.

Methods: Retrospective review of clinical data (age at onset, family history, clinical presentation, diagnostic delay, diagnosis) and *APOE* genotype of patients with neuropathologically confirmed EOAD (<60 years).

Results: Forty cases were selected. Mean age at onset was 54.5 years (range 46–60). The mean disease duration was 11 years with a mean diagnostic delay of 3.1 years. A total of 37.5% had a nonmemory presentation. Behavioral/executive dysfunction was the most prevalent atypical presentation. Incorrect initial clinical diagnoses were common (53%) in patients with atypical presentations, but rare when anterograde amnesia was the presenting symptom (4%). The incorrect initial clinical diagnoses were 2 behavioral variant frontotemporal lobar degeneration, 2 normal pressure hydrocephalus, 1 semantic dementia, 1 primary progressive aphasia, 1 corticobasal degeneration, 1 pseudodementia with depression, and 1 unclassifiable dementia. *APOE* genotype was $\epsilon 3/\epsilon 3$ in 59%, with no significant differences between typical and atypical presentations. *APOE* $\epsilon 4$ was 3.3 times more frequent in subjects with family history of AD. A total of 97.5% of the cases presented advanced neurofibrillary pathology. A total of 45% of the patients had concomitant Lewy body pathology although localized in most cases and without a significant clinical correlate.

Conclusion: One third of patients with pathologic confirmed EOAD presented with atypical symptoms. Patients with EOAD with nonamnestic presentations often receive incorrect clinical diagnoses. ***Neurology*® 2011;76:1720–1725**

GLOSSARY

AD = Alzheimer disease; **CBD** = corticobasal degeneration; **CBS** = corticobasal syndrome; **EOAD** = early-onset Alzheimer disease; **FTLD** = frontotemporal lobar degeneration; **LB** = Lewy body; **LOAD** = late-onset Alzheimer disease; **NPH** = normal pressure hydrocephalus; **NTB/UB/HC** = Neurological Tissue Bank, University of Barcelona-Hospital Clínic; **PCA** = posterior cortical atrophy; **PPA** = primary progressive aphasia; **SemD** = semantic dementia.

Alzheimer disease (AD) is the most frequent cause of degenerative dementia in developed countries.[1,2] The typical clinical pattern starts by episodic memory dysfunction and then progresses to other cognitive domains although atypical presentations, without episodic memory impairment at onset, have also been described.[3-6]

Although the pathologic changes are similar regardless the age at onset, AD has been divided into 2 clinical forms according to age at onset: early-onset AD (EOAD) and late-onset AD (LOAD). Several clinical series have shown that EOAD presents more frequently with atypical clinical manifestations such as visual, executive, behavioral, or language impairment compared with LOAD.[7] One limitation of clinical studies is that the diagnosis is made using clinical criteria, often lacking pathologic confirmation.[8]

Supplemental data at www.neurology.org

From the Alzheimer's Disease and Other Cognitive Disorders Unit (M.B., A.A., R.S.-V., J.L.M., A.L.), Neurology Service, Hospital Clínic, Institut d'Investigació Biomèdica August Pi i Sunyer (IDIBAPS), Barcelona; and Neurological Tissue Bank University of Barcelona/Hospital Clínic (NTB/UB/HC) (E.G., M.J.R.), Barcelona, Spain.

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The aim of our study was to describe the clinical features of a population of confirmed EOAD, investigate the frequency of nonmemory presentations in EOAD, and identify features that lead to misdiagnoses. We also sought the frequency of the different *APOE* genotypes and its correlation with their clinicopathologic phenotypes.

METHODS

Standard protocol approvals, registrations, and patient consents. All the brain donors fulfilling neuropathologic criteria of AD with age at onset before 60 years were selected from the Neurological Tissue Bank, University of Barcelona–Hospital Clínic (NTB/UB/HC) (1994–2009). All individuals or relatives had given their informed consent for research. We selected the age at onset before 60 years in order to assure the selection of early-onset cases. We excluded subjects with insufficient clinical information and carriers of pathogenic mutations in presenilin 1 (*PSEN1*), presenilin 2 (*PSEN2*), and amyloid precursor protein (*APP*) genes because our aim was to describe clinical profiles of nongenetic EOAD.

Clinical classification. We reviewed the medical records available at the NTB/UB/HC and also contacted the neurologists who attended the patients during life. They were asked to fill in a form with clinical information: age at onset, personal and family history, clinical presentation, cognitive domains affected at first evaluation, diagnostic delay (disease duration until the first diagnosis), initial clinical diagnosis, and final clinical diagnosis (at the last visit before death). Positive family history of disease was considered when at least one first-degree relative presented a clinical picture suggestive of dementia. An autosomal dominant pattern of inheritance was defined by the presence of at least 3 family members with dementia in 2 generations. The cognitive domains affected at first evaluation were assigned according to the opinion of the attending neurologist or the neuropsychological evaluation. In atypical presentations, mild memory impairment could not be excluded in some cases although it was not the main complaint of the patient.

Patients were classified into different clinical subtypes:

1. Typical presentation: episodic memory impairment with progression to other cognitive domains.
2. Atypical presentation (no episodic memory dysfunction at clinical onset): frontal variant (behavioral problems [inappropriate social conduct, impulsivity, disinhibition, apathy, or mood disturbances such as depression], predominant executive dysfunction); posterior variants (posterior cortical atrophy [PCA]: visuospatial problems in a patient with normal insight and no ocular disease that could explain the symptoms, components of Balint or Gerstmann syndromes, or progressive apraxic syndrome; corticobasal syndrome [CBS]: combination of asymmetric parkinsonism and cortical dysfunction signs [alien limb, limb apraxia, cortical sensory loss]); and language variant: aphasia as the first and principal symptom at onset.

Neuropathologic diagnosis. Neuropathologic examination was performed according to standardized protocols at the NTB/UB/HC. Half brain is usually fresh-frozen and stored at −80°C and the other half is fixed in formaldehyde solution. At least 25 representative brain areas are embedded in paraffin. For histologic evaluation, 5-μm-thick sections are stained with hematoxylin & eosin. Immunohistochemistry is performed using various antibodies including anti-βA4, anti-ptau, anti-RD3, anti-RD4, anti-α-synuclein, anti-ubiquitin, anti-α-internexin, and anti-TDP-43. Disease evaluation was performed according to international consensus criteria.[9,10] Semiquantitative assessment of tau neuropil threads, neurorofibrillary tangles, and β-amyloid deposits (+ mild, ++ moderate, +++ severe) was performed in frontal, temporal, parietal, occipital cortices, insula, and amygdala. The presence of tau inclusions in granular neurons of dentate gyrus, tau-positive grains, as well as of capillary cerebral amyloid angiopathy was recorded.[11-13]

Genetic analysis. DNA was extracted from cerebellum using the QIAamp DNA Minikit for DNA purification from tissues (QIAGEN Co.) following manufacturer's instructions. The *APOE* genotype was determined using PCR amplification and *Hha*I restriction enzyme. In one patient, the determination of *APOE* genotype was not possible due to lack of sample. Genetic analysis by direct sequencing of *PSEN1* (exons 3–12), *PSEN2* (exons 3–12), and *APP* (exons 16 and 17) genes was performed in patients with a positive family history.

Statistical analysis. For statistical analysis, SPSS version 16.0 was used. We used χ^2 test for categorical data and t tests for continuous data. Statistical significance was set at $p < 0.05$.

RESULTS

Demographic features, clinical classification, and genetics. From the initially 54 cases selected, 11 cases of genetic AD and 3 cases without reliable clinical data were excluded. Therefore, a total of 40 cases (25 male, 15 female) were included. Demographic, clinicopathologic, and *APOE* characteristics of the patients are summarized in table 1.

Twenty-five patients (62.5%) presented with typical episodic memory dysfunction as the first symptom. Their initial diagnosis was AD in 24 cases and normal pressure hydrocephalus (NPH) in the other case. In this group, 8 patients (32%) had clinically significant mood disturbances (depression) at onset.

The other 15 patients (37.5%) had an atypical presentation. Their clinical phenotypes were frontal variant (7 patients), 5 posterior variant (2 with predominant visuospatial dysfunction and 3 progressive apraxic syndrome/CBS), and language disturbances in 3 patients. Their initial clinical diagnoses were 2 AD, 2 PCA-AD, 3 AD vs frontotemporal lobar degeneration (FTLD), 2 behavioral variant FTLD, 1 semantic dementia (SemD), 1 primary progressive aphasia (PPA), 1 NPH, 1 corticobasal degeneration (CBD), 1 pseudodementia with depression, and 1 unclassifiable dementia (table e-1 on the *Neurology*® Web site at www.neurology.org). No significant differences were found between typical and atypical presentations in gender, mean age at onset, diagnostic delay, age at death, or disease duration (table 1).

Fourteen patients had a positive family history of dementia (with early onset in 4 cases) but none had an autosomal dominant pattern of inheritance as previously defined. No pathogenic mutations in

Table 1 Demographics, clinical characteristics, and *APOE* genotypes of the patients

	Total	Typical presentation	Atypical presentation	*p* Value
No. of patients	40	25	15	
Male	25	13	12	NS
Mean age at onset, y	54.5	54.7	54.2	NS
Diagnostic delay, y	3.1	3.2	3	NS
Age at death, y	65.5	66.16	64.4	NS
Total duration of disease, y	11	11.4	10.2	NS
APOE ε3/ε3, %	59	58.3	60	NS
APOE ε4/ε3, %	28.2	25	33.3	NS
APOE ε4/ε4, %	12.8	16.6	6.6	NS
Initial clinical misdiagnosis, %	22.5	4	53	0.0003
Final clinical misdiagnosis, %	20	4	47	0.0011
Presence of Lewy body (all localizations), %	45	40	46.6	NS

Abbreviation: NS = not significant.

PSEN1, *PSEN2*, and *APP* genes were identified. Sixteen out of 39 patients (41%) carried *APOE* ε4 allele.

Neuropathology. Fresh brain weight varied from 810 g to 1,410 g (mean of 1,092 g). All cases showed Alzheimer-type pathology. The breakdown by Braak stage was 32 cases with Braak stage VI (80%), 7 stage V (17.5%), and 1 stage IV (2.5%). All cases had a high density of neuritic plaques (score C of Consortium to Establish a Registry for Alzheimer's Disease criteria), and a high probability that the dementia was caused by Alzheimer pathology according to NIA/Reagan Institute criteria. A total of 87.5% had a phase 4 or 5 of amyloid deposits. A total of 18 patients (45%) showed variable degree of concomitant Lewy body (LB) pathology, with amygdalar LB in 9 patients (22.5%), limbic LB (Braak stage 4) in 5 patients (12.5%), and neocortical LB (Braak stage 5) in 3 patients (7.5%). Vascular lesions were observed in 12.5%, 2 patients had additional hippocampal sclerosis, 1 case combined AD pathology with motor neuron disease (TDP-43 positive), and in 1 case AD changes were accompanied by 4R tauopathy compatible with PSP. The distribution of tau and β-amyloid pathology in individual subjects in the different brain areas is shown in table e-2.

Description of some illustrative atypical cases (appendix e-1). ***Clinicopathologic and genetic correlation.*** Overall, the percentage of initial clinical misdiagnosis was 22.5%, which was maintained until the patient's death in 20% of the cases (table 1). Misdiagnosis was significantly higher in the atypical compared with typical clinical presentations, both at initial (53% vs 4%, χ^2 test $p = 0.0003$) and at final clinical diagnosis (47% vs 4%, χ^2 test $p = 0.0011$).

In the amnestic presentation subgroup there was a low percentage of clinical misdiagnosis: 1/25 (4%) did not have AD as final clinical diagnosis (one initial NPH diagnosis was changed to AD during follow-up and in one patient the final clinical diagnosis was dementia with Lewy bodies). In contrast, 7/15 (47%) of the nonamnestic presentation patients were not diagnosed with AD before death (2 behavioral FTLD, 2 CBD, 1 SemD, 1 FTLD with motor neuron disease, and 1 unclassifiable dementia).

No significant differences were found in *APOE* genotype between typical and atypical presentations (table 1). The *APOE* ε4 carriers were 3.3-fold more frequent in familial cases (76.9% vs 23.1%, confidence interval 1.5–7.1, χ^2 test $p = 0.001$). No significant differences were found between *APOE* ε4 carriers/noncarriers in mean age at onset, diagnostic delay, age at death, or disease duration.

No differences in the density of tau-positive neuropil threads, tangles, or β-amyloid deposits were found in frontal cortex as compared to other brain areas in the behavioral variant of AD compared to the other variants. We also did not find differences in the frequency of capillary CAA, tau-positive grains, or tau inclusions in neurons of dentate gyrus (table e-2).

There were no correlations between the presence of LB and clinical presentation, age at onset and death, sex, diagnostic delay, disease duration, or *APOE* genotype.

DISCUSSION In this retrospective study of pathologically confirmed patients with EOAD, we observed frequent misdiagnoses among patients with atypical (nonmemory) presentations. *APOE* ε4 was more frequent in familial cases but did not appear to have influenced the clinical phenotype. Although relatively high in absolute numbers, the coexisting pathology (mainly LB) lacked a clinical correlate.

Atypical presentations of EOAD represent a problem in everyday clinical practice because of its frequency[14] and the higher risk of misdiagnosis. Over half of the patients with EOAD with nonamnestic presentations were given etiologic diagnoses other than AD. The percentage of misdiagnoses was maintained at final clinical diagnosis with little changes. The misdiagnoses were mainly degenerative (FTLD group, CBD), but also nondegenerative pathologies (NPH and mood disorders).

It is well known by now that, in some cases, the clinical picture correlates poorly with the pathologic changes. One clear example could be the progranulin gene mutation which causes FTLD tau-negative ubiquitin-positive pathology and clinically had been

related to frontal variant of FTLD, progressive nonfluent aphasia, CBS, or even AD.[15]

A diagnostic delay of more than 3 years was observed, with no difference between the typical and atypical presentations. The diagnostic delay in EOAD might have been caused by the young age itself because the family and the attending physicians do not contemplate dementia in the initial differential diagnosis.

In the future, when there are more potent therapies for AD, it might be desirable to use AD biomarkers for the clinical diagnosis, such as CSF biomarkers or in vivo amyloid neuroimaging techniques, in routine clinical practice. However, at the present time, data about cost/effectiveness of these interventions is needed.[16-18]

The frontal variant, characterized by behavioral or dysexecutive problems, was the most frequent nonmemory presentation in our group.[19] Only 43% had the final clinical diagnosis of AD, with a similar percentage of FTLD clinical misdiagnoses. When a young patient exhibits a disturbance of personality and interpersonal relationships, the usual diagnosis is FTLD.[20] However, AD is a common cause of early-onset dementia and should always be considered as an alternative diagnosis.[1,2] One of our patients with advanced AD changes also had ubiquitin and TDP43-positive cytoplasmic inclusions in pyramidal neurons of frontal and primary motor cortex, motor neurons of hypoglossal nerve, dentate nucleus, and motor neurons of spinal cord. There were no TDP43 inclusions in temporal cortex or limbic system including the hippocampus. The clinical presentation was a frontal syndrome, developing memory impairment 3 years later. Nine years after clinical onset he presented aggressive motor neuron disease which led him to death. Although we cannot rule out mixed pathology, he had widespread AD pathology.

The posterior variant of AD comprises the syndromes of PCA and CBS. PCA is a well-recognized syndrome associated with AD pathology in the majority of cases,[21-23] with 2 clinical subtypes identified: the visuospatial variant (with prominent visuospatial symptoms) and the apraxic type (the biparietal syndrome, presenting with prominent apraxic symptoms associating also visuospatial problems).[24,25] The neuropathologic substrate of CBS is in most cases a 4-repeat tauopathy, although various clinicopathologic series showed that a significant percentage of patients with CBS had AD pathology.[26] We identified 5 patients with a posterior onset: 2 with prominent visuospatial disturbances (diagnosed correctly as having AD) and 3 with prominent apraxic syndrome with parkinsonism (in only one of them was AD pathology taken into consideration).

A progressive disturbance of language could be the first symptom of AD or FTLD. Several types of degenerative aphasias have been described. Two of them are classified into the group of FTLD: progressive nonfluent aphasia and semantic dementia.[20] A third type, logopenic aphasia, appears to be related with AD pathology. It is characterized by a decreased spontaneous speech production, frequent word-finding pauses, preservation of motor speech and grammar, and altered repetition.[27,28] From the 3 aphasic patients of our series, 2 were diagnosed with AD. Unfortunately, all 3 patients were seen in advanced stages and the neuropsychological evaluation did not help to categorize them more accurately.

At a pathologic level, we have found a significant percentage of coexistent pathology, mainly LB. Although the synuclein pathology could be considered as high, half of the LB were amygdalar, without involvement of other cerebral structures. Additionally, this finding did not change the pathologic diagnosis and did not have a statistically significant clinical correlate. We cannot rule out completely the presence of late-life hallucinations and the possible correlations with the presence of LB as we have rather poor clinical information on the last phases of the disease in most patients.

APOE ϵ4 is the most prevalent genetic risk factor for AD.[29,30] In our series, 41% of the patients were *APOE* ϵ4 carriers. There was no significant difference between the typical and atypical presentations in *APOE* genotypes. Other studies found different results in clinical cohorts,[31,32] showing that the percentage of ϵ4 carriers is lower in atypical AD cases than in memory-onset patients. This discrepancy could have several explanations: one might be that the previously reported data based on clinical criteria (without neuropathologic confirmation) might have overlooked some ϵ4 carriers in the atypical presentation group. It could also be postulated that the typical amnesic phenotype is strongly associated with the *APOE* ϵ4 allele, but our sample size was too small to detect a relationship.

The *APOE* ϵ4 allele was 3.3 times more frequent among subjects with a first-degree relative affected, suggesting that *APOE* ϵ4 could account for the familial aggregation of those cases, taking into account that the usual genetic causes of AD had been excluded from our series.

Our study has some limitations. First, the retrospective gathering of data could limit the accuracy of some clinical details, especially at the final clinical stages. Second, a selection bias is possible because the clinicians could be more prone to promote brain donation in atypical cases.

AUTHOR CONTRIBUTIONS

M. Balasa and A. Lladó conceived the study. M. Balasa, A. Lladó, J.L. Molinuevo, R. Sanchez-Valle, and all the other members of the NTB/UB/HC collaborative group listed as coinvestigators contributed to subject recruitment. M. Balasa and A. Lladó gathered the clinical data. E. Gelpí and M.J. Rey gathered and reviewed the pathologic data. M. Balasa and A. Antonell performed the DNA extraction and *APOE* determination. A. Antonell performed the genetic study of *PSEN1*, *PSEN2*, and *APP*. M. Balasa and A. Lladó wrote the first draft of the manuscript. M. Balasa performed the statistical analysis. M. Balasa, A. Antonell, E. Gelpí, M.J. Rey, R. Sanchez-Valle, J.L. Molinuevo, A. Lladó, and the other members of the NTB/UB/HC collaborative group listed as coinvestigators provided critical comments and contributed to the discussions/ A. Lladó provided overall supervision of the study.

COINVESTIGATORS

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DISCLOSURE

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Appendix e-1: Description of some illustrative atypical cases

(see Table e-1 for all non-memory onset cases)

Case 6: Behavioral alterations/Frontal variant

This 62-year-old, right-handed salesman was referred for behavioral disturbances that had started 4 years earlier. His family reported bluntness, lack of hygiene, inappropriate social conduct, apathy, and loss of interest in his work and hobbies. At first evaluation he scored a Minimental State Examination (MMSE) of 27. His CT scan revealed global brain atrophy, and perfusion SPECT showed bilateral temporal hypoperfusion. Behavioral variant FTLD was diagnosed. The behavioral abnormality increased during follow-up; he developed paranoid and violent conduct and a progressive aphasic syndrome leading to mutism at age 64 years. He died at the age of 66. Neuropathological examination showed ubiquitous advanced Alzheimer type pathology combined with neocortical LB (Braak stage 5). (See table e-2 for tau and beta-amyloid pathology distribution in all cases.)

Case 10: Corticobasal syndrome

A 60-year-old illiterate right-handed woman presented with a 4-year history of mood and motor disturbances. Her mother had late-onset AD. At first neurological examination she scored a MMSE of 22 and had ideomotor apraxia and a left-sided parkinsonism. MRI showed asymmetric brain atrophy (predominantly on the right side) and perfusion SPECT revealed bifrontal and right temporal hypoperfusion. Levodopa was prescribed without improvement. Corticobasal degeneration was diagnosed. The apraxic and parkinsonian

syndrome worsened in the following years and prominent behavioral disturbances appeared. She never presented visual hallucinations. She died at age 64 years. Neuropathology demonstrated advanced AD changes associated with cortical LB (Braak 5).

Case 11: Posterior cortical atrophy

This 53-year-old left-handed factory worker presented with a 2-year history of visual problems. He had problems recognizing letters/numbers and following the lines while reading. He also reported apraxic problems like buttoning the shirt and difficulties in driving. At the neuropsychological testing he presented important right hemisphere dysfunction with left-side neglect, MMSE of 24, mild episodic memory dysfunction, and no language problems. There was parieto-occipital atrophy in the MRI; the perfusion SPECT revealed marked temporoparietal hypoperfusion (more on the right side). He was diagnosed as having PCA (probable AD). He died at the age of 60. Neuropathological examination showed marked Alzheimer-type pathology with small vessel disease and lacunar infarcts in the basal ganglia.

Case 15: Language alteration

A 54-year-old salesman, right-handed, was referred for a progressive aphasic syndrome with marked anomia, altered comprehension, and correct grammatical structure. A formal neuropsychological evaluation was not performed because of the language impairment. Cerebral perfusion SPECT showed bilateral hypoperfusion of temporal poles and medial temporal region, and global atrophy on the MRI more pronounced on the anterior temporal lobes. SemD was diagnosed. After 4 years he developed prosopagnosia and progressive episodic memory failure. The diagnostic of SemD was maintained throughout his entire life. He died at age 57. Neuropathology showed diffuse advanced Alzheimer-type changes with severe cerebral amyloid angiopathy.

Table e-1. Patients with atypical presentations

	Age at onset/Age at death(years)/ Sex (F/M)	Clinical syndrome at onset	Initial clinical diagnosis	APOE	Final clinical diagnosis	Pathology
1	59 / 68 / F	Mood disturbances	Pseudodementia with depression	ε4/ε3	AD	NFT Braak VI;CERAD frequent Phase 5 amyloid; Marked CAA
2	56 / 67 / M	Mood disturbance	AD	ε4/ε3	AD	NFT Braak IV; CERAD frequent; Phase 4 amyloid; Low frequency CAA Hippocampus sclerosis
3	58 / 67 / M	Abnormal social conduct, mood disturbances	Bv-FTLD	ε4/ε4	Bv-FTLD	NFT Braak VI; CERAD frequent; Phase 5 amyloid; Moderate CAA; Amygdalar Lewy bodies
4	58 / 68 / M	Anxiety crisis/Progressive executive dysfunction	AD	ε3/ε3	AD	NFT Braak VI; CERAD frequent; Phase 5 amyloid; Marked CAA; Limbic Lewy bodies (Braak 4)
5	51 / 69 / F	Paranoid ideas then progressive aphasic-apraxic–agnosic syndrome	Unclassifiable dementia	ε4/ε3	Unclassifiable dementia	NFT Braak VI; CERAD frequent; Marked CAA; Phase 5 amyloid; Limbic Lewy bodies (Braak 4); Vascular lesions basal ganglia
6	58 / 66 / M (see text for details)	Inappropriate social behaviour, lack of hygiene	Bv-FTLD	ε3/ε3	Bv-FTLD	NFT Braak V; CERAD frequent; Moderate CAA; Cortical Lewy bodies (Braak 5)

7	49 / 58 / M	Behavioural/ Executive dysfunction*	AD versus Bv-FTLD	ε3/ε3	FTLD with motor neuron disease	NFT Braak VI; CERAD frequent; Stage 5 amyloid; Moderate CAA; ALS TDP43+
8	58 / 68 / M	Gerstmann syndrome **	AD versus CBD	ε3/ε3	AD versus CBD	NFT Braak V; CERAD frequent
9	52 / 62 / M	Apraxic syndrome with progressive walking and urinary disfunction***	NPH	ε3/ε3	CBD	NFT Braak VI; CERAD frequent; Stage 5 amyloid; Low frequency CAA
10	56 / 64 / F (see text for details)	Mood disturbance and asymmetric parkinsonian syndrome	CBD	ε3/ε3	CBD	NFT Braak V; CERAD frequent; Stage 4 amyloid; Low frequency CAA; Cortical Lewy bodies (Braak 5)
11	51 / 60 / M (see text for details)	Visuospatial	PCA (AD)	ε3/ε3	PCA (AD)	NFT Braak VI; CERAD frequent; Stage 5 amyloid; Moderate CAA, Lacunar state
12	46 / 56 / M	Visuospatial and Apraxic syndrome	PCA (AD)	ε4/ε3	PCA (AD)	NFT Braak VI; CERAD frequent; Stage 5 amyloid; Pseudo/cotton wool plaques; Marked CAA; Amygdalar Lewy bodies
13	56 / 67 / M	Fluent aphasia	PPA	ε3/ε3	AD	NFT Braak VI; CERAD frequent; Stage 5 amyloid; Moderate CAA
14	53 / 69 / F	Mixed aphasia, visuospatial dysfunction	AD versus FTLD	ε3/ε3	AD versus FTLD	NFT Braak VI; CERAD frequent; Stage 5 amyloid; Low intensity CAA; Amygdalar Lewy bodies
15	52 / 57 / M (see text for details)	Fluent aphasia	SemD	ε4/ε3	SemD	NFT Braak VI; CERAD frequent; Stage 5 amyloid; Marked CAA

<u>Supplementary data:</u> Patients with atypical presentations

AD - Alzheimer disease; ALS – Amiotrophyc lateral sclerosis; Bv-FTLD - Behavioural variant-Frontotemporal lobar degeneration; CAA - Cerebral amyloid angiopathy; CBD - Corticobasal degeneration; CERAD - Consortium to Establish a Registry for Alzheimer's Disease; FTLD - Frontotemporal lobar degeneration; NFT – Neurofibrillary tangles; NPH - Normal pressure hydrocephalus; PCA - Posterior cortical atrophy; SemD - Semantic dementia;

* During follow-up presented overt motor neuron disease which lead to diagnose FTLD with motor neuron disease.
** Dysgraphia /Acalculia / Left-right disorientation. Apraxic syndrome
*** Dressing apraxia/Agrafia/After 1 years appeared walk disturbance and urinary incontinence. No improvement with ventricular peritoneal shunt. Asymetric parkinsonism during follow-up

Table e-2. Distribution of Tau and Beta-amyloid pathology in the clinical subgroups.

	Case number	Tau density								Abeta-density						
		Frontal	Temporal	Parietal	Occipital	Insula	Amygdala	Dentate gyrus	Grains	Frontal	Temporal	Parietal	Occipital	Insula	Amygdala	CAA
Frontal variant																
	1	++	++	++	+	++	+++			++	++	++	++	++	+	+++
	2	+	+	+/0	+/0	+/0	+++	yes		++	++	++	++	++	++	+ focal
	3	+++	+++	+++	++	+++	++	yes		+++	+++	+++	++, cCAA	+++	+++	+++, cCAA
	4	+++	+++	+++	+	+++	+++			+++	++	+++	++	+++	++	+++
	5	+++	+++	+++	+++	+++	+++	yes	yes	+++	+++	++	+++	+++	++	++++
	6	+++	+++	+++	+/0	+++	+++	yes		++	++	+++	++, cCAA	+++	++	++, cCAA
	7	+	++	+++	+/0	++	+++	yes		+++	+++	+++	++	+++	++	+ focal
Posterior variant																
	8	+	++	++	+/0	+	++			+++	++	++	++	++	++	++, focal
	9	+++	+++	+++	++	++	+++	Isolated		+++	+++	+++	++	+++	++	+ focal
	10	+	++	++	+/0	++	++			++	++	++	++	++	++	+ focal
	11	++	+++	+++	+	++	+++			++	++	++	++	++	++	+ focal
	12	+++	+++	+++	+++	+++	+++	Isolated		+++	++	++	++	++	++	+++
Language variant																
	13	++	+++	+++	+	++	+++			+++	++	+++	++	++	++	++
	14	+++	+++	+++	+++	+++	+++	Yes		+++	+++	+++	++	+++	++	+ focal
	15	+++	+++	+++	+++	+++	+++	Isolated		++, cCAA	++, cCAA	++, cCAA	++, cCAA	++	++	+++, cCAA
Amnestic variant																
	16	+++	+++	+++	+++	+++	+++			+++	++	+++	+++	++	++	+++
	17	++	++	+++	++	++	+++			+++	++	++	++	+++	++	++
	18	+++	+++	+++	+++	+++	+++			+++	++	+++	++	+++	++	no
	19	++	+++	++	+/0	++	++			++	++	++, cCAA	++, cCAA	+++	++, cCAA	+++, cCAA
	20	++	++	++	+	++	+++			+++	++	++	++	+++	++	++

	21	+++	+++	+++	+++	+++	+++	Yes		+++	+++	+++	++	+++	++	+++
	22	+++	++	na	na	na	++			+++	++	na	na	na	++	+++
	23	++	+++	++	++	++	++			+++	++	+++	++	+++	++	+++
	24	++	++	++	+++	++	++			++	++	+++	++	++	++	+ focal
	25	+++	+++	+++	+++	+++	+++	Yes	yes	++	+++	++	++, cCAA	+++	++	++, cCAA
	26	++	++	++	+	na	++			+++	++	++/+++	++	na	++	++
	27	+++	+++	+++	++	+++	+++	Yes	yes	++	++	++	++, cCAA	++	++	+++, cCAA
	28	++	++	na	+/0	++	++			++	++	++	++	++	++	+ focal
	29	++	+++	+++	+	++	+++			++	++	+++	+++	++	++	+ focal
	30	+++	+++	+++	+++	+++	+++	Yes		++	++	+++	++	++	++	++
	31	+++	+++	+++	+	++	++	Isolated		++	++	++	++	++	++	++
	32	++	++	++	++	++	++	Isolated		+++	++	++	++	++	++	++
	33	+++	+++	+++	++	+++	++	Yes	yes	++	++	++	++	+++	++	++
	34	+++	+++	+++	+++	+++	+++	Yes		+++	+++	+++	++	+++	++	+ focal
	35	++	++	++	+/0	++	++	Yes		+++	++	++	++	++	++	++
	36	+++	++	+++	++	+++	+++			++	+++	++	++	++	+++	++
	37	+	++	+	+	+	++			++	++	++	++	+++	++, cCAA	+++
	38	+++	+++	+++	++	++	+++	Yes		++	++	++	++	++	++	++
	39	+++	+++	+++	+/++	++	+++			++	++	++	++	++	++	+ focal
	40	+++	+++	+++	+++	+++	+++	Yes		+++	++	+++	++	+++	++	+ focal

CAA - cerebral amyloid angiopathy
cCAA – capillary cerebral amyloid angiopathy

Trabajo número 2

PSEN1 mutation carriers present lower cerebrospinal fluid amyloid-β_{42} levels than sporadic early-onset Alzheimer's disease patients but no differences in neuronal injury biomarkers.

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RESULTADOS

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PSEN1 Mutation Carriers Present Lower Cerebrospinal Fluid Amyoid-β_{42} Levels than Sporadic Early-Onset Alzheimer's Disease Patients but no Differences in Neuronal Injury Biomarkers

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Abstract. Most cases of early-onset Alzheimer's disease (EOAD) are sporadic. A minority of EOAD are caused by specific genetic defects in *PSEN1*, *PSEN2,* or *AβPP* genes. Magnetic resonance imaging (MRI) and cerebrospinal fluid (CSF) biomarker comparisons between sporadic and monogenic EOAD are practically inexistent. CSF and MRI data from 14 amnestic-onset sporadic EOAD (sEOAD) subjects were compared with data from 8 symptomatic *PSEN1* mutation carriers (PSEN1) and 14 age-matched cognitively-preserved controls. CSF concentrations of amyloid-β $(A\beta)_{42}$, total tau (t-tau), and phosphorylated tau (p-tau) were determined. Cortical thickness (CTh) and grey matter loss were compared between groups and correlated with CSF biomarkers. PSEN1 had significantly lower CSF $A\beta_{42}$ levels compared to sEOAD (mean 244.8 pg/ml versus 381.4 pg/ml; $p = 0.006$), but no differences in t-tau or p-tau. Both sEOAD and PSEN1 showed widespread CTh loss in AD target areas when compared with controls. No differences were found in the direct comparison between sEOAD and PSEN1 CTh after adjusting for age and Mini-Mental Status Examination scores. Neither was a correlation found between $A\beta_{42}$ levels and CTh. CTh in the left superior parietal and caudal middle frontal areas was negatively correlated with t-tau values. In conclusion, PSEN1 had lower $A\beta_{42}$ CSF levels compared with sEOAD, suggesting a greater cerebral deposition of $A\beta_{42}$.

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These differences in $A\beta_{42}$ deposition were not significantly reflected in the brain structure, and CTh was only correlated with total tau. The lack of significant differences in relation to t-tau and p-tau levels and to the severity of CTh or grey matter loss suggests a similar level of neuronal injury despite higher $A\beta_{42}$ load in PSEN1.

Keywords: $A\beta_{42}$ levels, Alzheimer's disease, cerebrospinal fluid biomarkers, early onset, *PSEN1* mutation

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INTRODUCTION

Alzheimer's disease (AD) is, in the majority of cases, a sporadic disease affecting subjects over 65 years of age. However, about 10% of cases have an earlier onset and initiate symptoms before the age of 65 (early-onset AD cases, EOAD). EOAD shares the main pathological findings with the late-onset variant of the disease (LOAD), namely neurofibrillary tangles composed of hyperphosphorylated tau protein and plaques formed by amyloid-β (Aβ) fibrils. A specific cerebrospinal fluid (CSF) profile, characterized by reduced $A\beta_{42}$ and increased total tau (t-tau) and phosphorylated tau (p-tau) has been described in AD, and this 'biological signature' has been widely replicated in both LOAD and EOAD [1, 2]. Comparisons between LOAD and EOAD made using clinical [3, 4] and neuroimaging data [5, 6] have shown greater impairment in attention, language, visuo-spatial and executive functions in EOAD, with LOAD having greater impairment in episodic memory. EOAD presents with more cortical atrophy and hypometabolism in widespread cortical areas encompassing frontal parietal and lateral temporal regions than does LOAD, although these differences seem to bear no relation to the amyloid load [5].

In fewer than 1% of cases, AD is transmitted with an autosomal dominant pattern of inheritance due to mutations in three genes: presenilin 1 (*PSEN1*), presenilin 2 (*PSEN2*), and amyloid-β protein precursor (*AβPP*). *PSEN1* mutations are the most frequent cause of autosomal dominant transmitted AD. To date, 185 mutations have been described in multiple families of diverse ethnic origin around the world [7]. The clinical onset in *PSEN1* mutation carriers (PSEN1) is, with a few exceptions, presenile [8, 9], and although genetic cases have, on occasions, some distinctive features the main clinical and pathological characteristics seem to be similar to those of sporadic disease [9–11]. PSEN1 carriers show the typical 'AD biological signature' in CSF analysis. However, quantitative studies comparing the CSF biomarkers of sporadic EOAD (sEOAD) and genetically-determined EOAD cases are lacking.

Recently, semi-automatic methods of magnetic resonance imaging (MRI) processing have afforded the opportunity of less time-consuming and operator-dependent analysis [12]. These methods, such as the surface-based measurement of cortical thickness (CTh) across the entire cortex, are able to compare vast cortical regions between groups in less time and with greater inter-operator reliability. However, few data are as yet available regarding the possible difference in cortical loss patterns between sEOAD and PSEN1.

The aim of the present study was to analyze differences in CSF biomarkers ($A\beta_{42}$, t-tau, and p-tau) between PSEN1 and sEOAD and to compare CTh maps or grey matter loss in these two groups. As a secondary objective we investigated the correlation between CSF biomarkers and cerebral structure in these early-onset AD subjects (both genetic and sporadic), given that the current hypothesis regarding the differential effect of $A\beta_{42}$ and tau on brain structure in the dementia phase of the disease has so far only been tested in LOAD.

MATERIALS AND METHODS

Subjects

Participants were recruited from the Alzheimer's Disease and Other Cognitive Disorders Unit at the Hospital Clínic, Barcelona, Spain. All participants gave written informed consent. The study was approved by the hospital's Ethics Committee.

Eight symptomatic patients carrying 6 different *PSEN1* mutations (M139T, L286P, K239N, L235R, L282R, I439S) were selected from the genetic counselling programme for familial dementias (PICOGEN) [8, 10, 13, 14]. They all had a typical amnestic onset of symptoms and fulfilled NIA/Alzheimer's Association criteria for probable AD dementia in a carrier of a causative AD genetic mutation [15] and Dubois et al. criteria for AD [16] at a stage of mild or moderate dementia.

Fourteen consecutive sEOAD subjects with a prototypical amnestic clinical presentation and an age at

clinical onset below 65 years were also included. They all fulfilled NIA/Alzheimer's Association criteria for probable AD dementia with evidence of AD pathophysiological process [15] and Dubois et al. criteria for AD [16] at a stage of mild to moderate dementia. An AD-compatible CSF profile was required in order to be included in the sEOAD group. None of these cases had a family history of EOAD. In order to ensure the two study groups were homogeneous, we only enrolled amnestic onset patients.

An age-matched control group of fourteen subjects with no cognitive complaints and no evidence of cognitive impairment was also selected.

Demographic (gender, age at the moment of MRI), clinical, and cognitive data were collected from all participants.

CSF analysis

All patients (except one PSEN1 and one control) underwent a lumbar puncture in the morning. The samples were centrifuged and stored in polypropylene tubes at -80°C within 2 h. Levels of $A\beta_{42}$, t-tau, and p-tau were measured using commercial sandwich ELISA kits (Innogenetics, Gent, Belgium). The cut-off values provided by the manufacturer, confirmed by reference papers and our internal data analysis, were 500 pg/ml for $A\beta_{42}$ and 75 pg/ml for p-tau. For t-tau the cut-off values were adjusted by age as follows: <50 years of age: 300 pg/ml; 51–70 years: 450 pg/ml [17, 18]. The laboratory in which the samples were processed is a participant in the QC programme and all values were within mean QC programme values ± 2 SD [19].

Magnetic resonance imaging acquisition

A high-resolution, three-dimensional, structural dataset (T1-weighted magnetization-prepared rapid gradient-echo, sagittal plane acquisition, repetition time = 2300 ms, echo time = 2.98 ms, 240 slices, field of view = 256 mm; matrix size = 256 × 256; slice thickness = 1 mm) was acquired in a 3T magnetic resonance imaging MRI scanner (Magnetom Trio Tim, Siemens Medical Solutions, Erlangen, Germany).

Cortical thickness procedure

Cortical reconstruction was performed with the FreeSurfer image analysis suite, version 4.5 (http://surfer.nmr.mgh.harvard.edu). The procedures have been described previously [20]. Reconstructed and registered individual CTh maps were smoothed using a Gaussian kernel of 15 mm full-width at half maximum and introduced into a group analysis based on general linear modeling (GLM) of the data in order to obtain spatial maps showing the differences between groups. The FreeSurfer streamline provided a cortical parcellation based on a predefined atlas [12]. Regions showing significant differences between groups were used for defining regions of interest (ROI). Mean thicknesses of the ROIs were extracted from each subject based on the aforementioned parcellations, and between-group comparisons and correlation analyses with CSF were performed.

Subcortical volume analysis

A volume-based subcortical segmentation was also performed with FreeSurfer. As described in Fischl et al. [21], the process consists mainly of: 1) an affine registration with Talairach space; 2) an initial volumetric labeling; 3) correction of the variation in intensity due to the B1 bias field; 4) performance of a high-dimensional, nonlinear volumetric alignment to the Talairach atlas; and 5) definitive labeling of the structures. The segmentation procedure also allows measurement of the estimated intracranial volume (EIV) [22], which is based on the scale factor used for atlas registration.

Voxel-based morphometry (VBM)

Structural data were also analysed with FSL-VBM, a VBM-style analysis (http://www.fmrib.ox.ac.uk/fsl/, Version 4.1.5). First, structural images were extracted using BET [23], before carrying out tissue-type segmentation with FAST4 [24]. Non-linear transformation of the native grey matter images into MNI152 standard space was then performed [25, 26]. The resulting images were averaged to create a study-specific template, to which the native grey matter images were then non-linearly re-registered. The registered partial volume images were then modulated (to correct for local expansion or contraction) by dividing by the Jacobian of the warp field. The modulated segmented images were then smoothed with an isotropic Gaussian kernel with a full width at half-maximum (FWHM) of 9 mm. Finally, voxel-wise GLM was applied using permutation-based non-parametric testing, correcting for multiple comparisons across space.

Statistical analysis

Difference maps for the between-groups CTh analyses were obtained using linear modeling of the CTh, as implemented in FreeSurfer, with age (and Mini-Mental Status Examination (MMSE) when specified) as covariates. The uncorrected results were thresholded at the $p < 0.01$ level, while the correction for multiple comparison consisted in a Monte Carlo Null-Z simulation performing 10000 iterations (family-wise error [FWE]). Only corrected clusters with a final cluster-wise probability <0.05 were considered. FreeSurfer was also used when correlating CTh and CSF values.

VBM comparisons between groups were obtained using GLM analysis with age as the covariate (and MMSE when specified). Uncorrected results were considered at $p < 0.001$, while corrected multiple comparison results were considered significant at $p < 0.05$, after implementing a threshold-free clustered enhancement [27].

Group analyses of demographic and CSF data were performed using the PASW 18.0 statistical package (IBM Corp.). Mann-Whitney U or Kruskal-Wallis tests were used for continuous variables. Correlation analysis was also performed between continuous variables using Spearman's Rho correlation coefficient. When not specified the data are presented as mean (SD).

RESULTS

Demographic and clinical characteristics

Demographic and CSF data are summarized in Table 1. PSEN1 were younger than the sEOAD group (48.9 versus 58.1 years, $p = 0.024$), but there were no significant age differences between controls and sEOAD or PSEN1. There were no significant differences in gender or APOE distribution, MMSE scores, or clinical disease duration between sEOAD and PSEN1.

CSF analysis

Both PSEN1 and sEOAD subjects (1 PSEN1 was not tested) had the typical AD CSF profile. After adjusting for MMSE, PSEN1 had significantly lower mean levels of CSF $A\beta_{42}$ compared with sEOAD (244.8 pg/ml (SD: 134.2) versus 381.4 (SD: 74.6), $p = 0.006$) (Fig. 1). There were no differences between the two groups in the mean levels of t-tau or p-tau, neither in the raw comparison nor after adjusting for MMSE and age (supplementary Figures 1 and 2; available online: http://www.j-alz.com/issues/30/vol30-3.html#supplementarydata03). Compared with controls, both PSEN1 and sEOAD had lower mean levels of CSF $A\beta_{42}$ and higher t-tau and p-tau levels (Table 1). No significant correlation was observed between CSF levels of $A\beta_{42}$, t-tau, p-tau, and clinical disease duration in PSEN1 and sEOAD.

MRI analysis

The EIV did not differ between groups (supplementary Table 1). Consequently, EIV was not included in the subsequent statistical models.

Cortical thickness analysis

Both sEOAD and PSEN1 had widespread cortical thinning compared with controls. The differences are described adjusting for age and after FWE correction for multiple comparisons ($p < 0.05$) (Fig. 2). The thinning pattern encompassed the posterior cingulate cortex (PCC), parietal and temporal areas such as the precuneus (PPC), the inferior and superior parietal cortex (IP and SP), the supramarginal cortex (SMC), the middle and superior temporal cortex (MT and ST), the bank of the superior temporal sulcus (BSTS), the parahippocampal gyrus and the lateral occipital cortex (LOC). In frontal areas the difference maps were less severe and restricted to certain areas such as the

Table 1
Demographic, clinical and CSF data of the subjects. Data are presented as mean (standard deviation). PSEN1 = symptomatic *PSEN1* mutation carriers; sEOAD = sporadic early-onset AD; CTR = controls

	CTR	sEOAD	PSEN1	CTR versus sEOAD	CTR versus PSEN1	sEOAD versus PSEN1
Age	54.0 (9.18)	58.1 (3.81)	48.9 (7.53)	$p = 0.343$	$p = 0.280$	$p = 0.024$*
Males (%)	28.6%	28.6%	37.5%	$p = 1$	$p = 0.6$	$p = 0.6$
APOE 4+	15.4%	64.3%	12.5%	$p = 0.03$*	$p = 1$	$p = 0.057$
Clinical duration (y)		2.3 (1.6)	3 (2.1)			$p = 0.4$
MMSE	28.9 (1.04)	23.4 (4.07)	19.6 (6.21)	$p = 0.004$*	$p < 0.001$*	$p = 0.119$
$A\beta_{42}$	682.65 (140.94)	381.41 (74.56)	244.83 (134.2)	$p < 0.001$*	$p < 0.001$*	$p = 0.006$*
t-tau	247.91 (77.45)	833.91 (395.36)	984.47 (562.28)	$p = 0.001$*	$p = 0.001$*	$p = 0.640$
p-tau	52.22 (11.00)	114.68 (27.99)	110.78 (57.19)	$p < 0.001$*	$p = 0.002$*	$p = 0.792$

*$p < 0.05$.

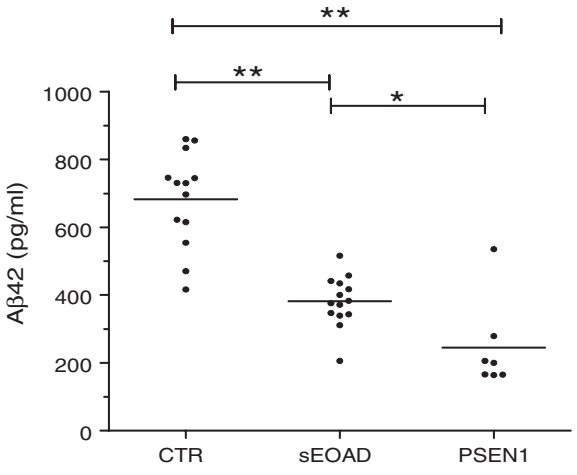

Fig. 1. Distribution of CSF $A\beta_{42}$ values in the different study groups. PSEN1 = *PSEN1* mutation carriers; sEOAD = sporadic early-onset AD; CTR = controls $*p < 0.01$, $**p < 0.001$.

caudal middle frontal (CMF) and superior frontal cortex (SFC) (supplementary Table 3).

The direct comparison between sEOAD and PSEN1 was carried out with age as a nuisance factor. The uncorrected ($p < 0.01$) difference map only showed small differences in CTh (Fig. 3), and these differences disappeared after adding MMSE as a nuisance variable and FWE correction. A *t*-test of CTh values for ROIs showing statistical differences in the uncorrected maps was performed and revealed no differences.

Voxel-based morphometry analysis

The VBM comparison between controls and AD groups (PSEN1 and sEOAD) showed a similar pattern of grey matter loss to that described above in the CTh analysis. The comparisons sEOAD-controls and PSEN1-controls had a common, extensive significant cluster (in green in Fig. 4) encompassing areas such as the SP, PPC, MT, ST, the LOC, the parahippocampal and fusiform gyrus and the insula. The hippocampus and the occipital and frontal cortex (particularly when the control group was compared to sEOAD) were also affected (Fig. 4 and supplementary Table 2). The thalamus seemed more affected in PSEN1.

In direct comparisons, PSEN1 had more grey matter loss than did sEOAD in the right and left thalamus, left and right ST cortex, right SFC, left and right PCC and left insula (supplementary Table 2), when adjusting only by age and at the uncorrected level ($p < 0.001$). However, multiple comparison adjustment showed no significant differences between sEOAD and PSEN1 in grey matter loss.

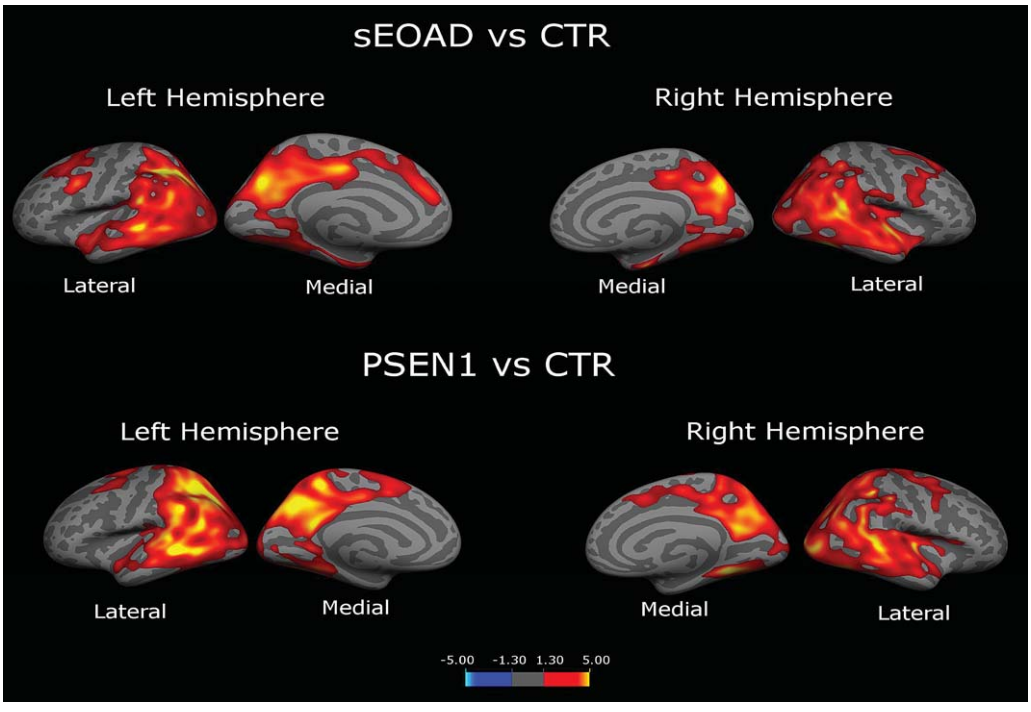

Fig. 2. Cortical thickness (CTh) difference maps. The upper figure represents the sEOAD and the lower the PSEN1 group, compared with a control group at $p < 0.05$ adjusting for age and after FWE correction for multiple comparison. Red-yellow areas represent decreased CTh.

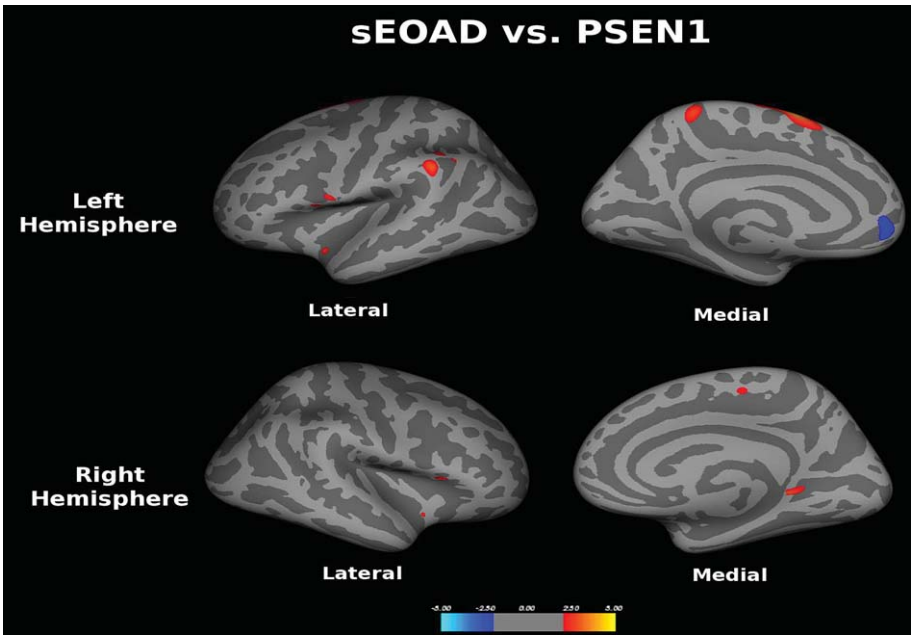

Fig. 3. Cortical thickness (CTh) difference maps for the PSEN1 versus sEOAD comparison at $p < 0.01$ uncorrected. Red-yellow areas indicate decreased CTh in PSEN1 with respect to sEOAD, while blue represents decreased CTh in sEOAD with respect to PSEN1.

Subcortical volume analysis

Given that VBM analysis indicated possible differences (albeit non-significant) between AD groups, especially in subcortical structures, a volume-based subcortical stream analysis was performed, comparing thalamic and hippocampal volumes between groups. No significant difference was identified between PSEN1 and sEOAD at baseline or after adjusting for age and MMSE (supplementary Table 1).

Relationship between CSF biomarkers and cortical thickness

There were no significant correlations between $A\beta_{42}$ and CTh when considering the PSEN1 and sEOAD groups individually. The same negative results were obtained when considering all AD patients as a single group. When correlating t-tau with CTh in the whole AD sample, two regions in the left hemisphere resisted FWE correction (Fig. 5): a SP cluster that also included a region of the PCC and ISTC, and a CMF cluster that also encompassed structures in the SFC and precentral cortex. We then checked the correlation between the mean CTh values of these predefined ROIs (left CMF and left SP atlas ROIs) and the individual t-tau CSF values (supplementary Table 3). Both ROIs showed a significant inverse correlation with t-tau levels ($r = -0.455$ ($p = 0.038$), and $r = -0.455$ ($p = 0.038$), respectively).

No correlation between p-tau and CTh was maintained after FWE correction.

DISCUSSION

This study investigated differences in CSF biomarkers and structural MRI between sEOAD and PSEN1. PSEN1 had significantly lower levels of $A\beta_{42}$ compared with sporadic cases. Despite these $A\beta_{42}$ differences, however, the pattern of CTh and grey matter loss was broadly the same in PSEN1 and sEOAD, and small areas with different CTh or grey matter volume loss were not maintained after adjusting for age and MMSE. CTh was inversely correlated with t-tau levels in the left caudal middle frontal and superior parietal cortex, but no structural correlations were found with $A\beta_{42}$ or p-tau.

$A\beta_{42}$ has been shown to be the AβPP subspecies that is most prone to amyloid fibril formation [28, 29]. $A\beta_{42}$ brain deposits are an early and constant feature of AD, appearing years before the first symptom [30] as a result of an imbalance between amyloid production and clearance. The different PSEN1 mutations affect γ-secretase function in different ways to produce a lifetime increase of the $A\beta_{42}/A\beta_{40}$ ratio [31, 32]. By contrast, in sporadic disease the main pathogenic

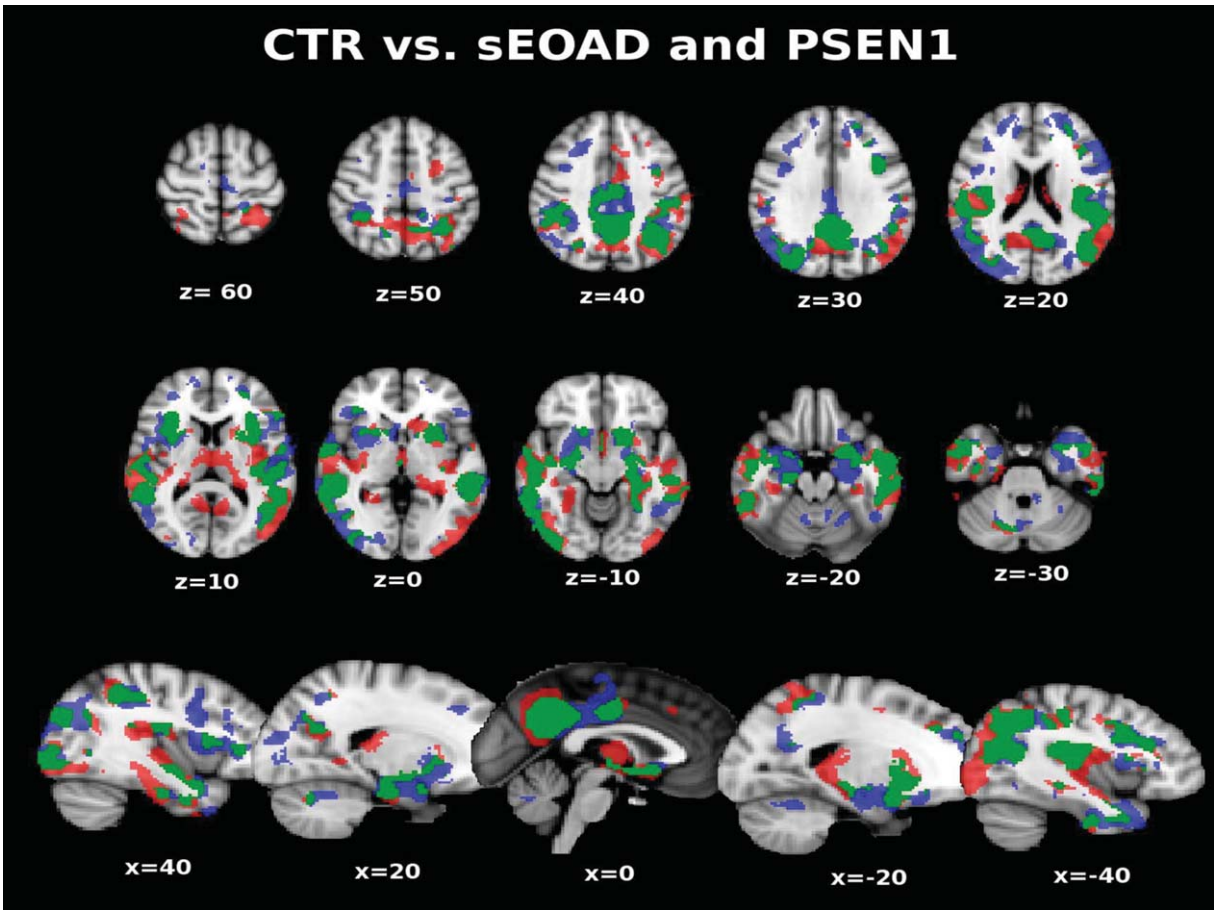

Fig. 4. Voxel-based morphometry (VBM) comparisons between controls and AD groups (PSEN1 and sEOAD). The results are shown after FWE correction with threshold-free cluster enhancement (TFCE) at $p < 0.05$. The coordinates correspond to the MNI atlas. The red color represents the CTR > PSEN1 comparison, the blue represents the CTR > sEOAD comparison and the green represents areas that are common to the two comparisons. PSEN1 = *PSEN1* mutation carriers; sEOAD = sporadic early-onset AD; CTR = controls.

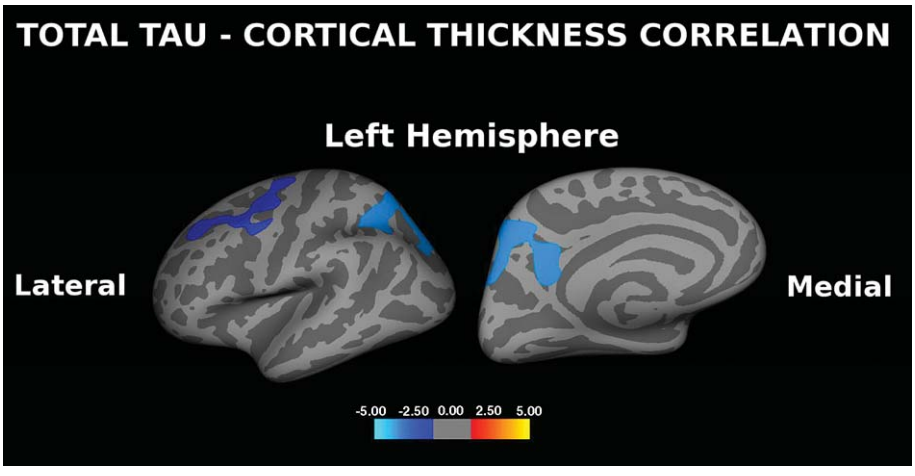

Fig. 5. Cortical thickness correlation map for CSF t-tau values (after FWE correction and at a threshold of $p < 0.05$). Blue/light blue colour represents a negative correlation.

mechanism seems to be a diminished clearance of Aβ [33].

Different studies have shown that diminished levels of CSF $A\beta_{42}$ reflect $A\beta_{42}$ deposition in brain [34]. In this regard, CSF $A\beta_{42}$ levels decrease in the pre-symptomatic phase of AD, reflecting the progressive parenchymal deposition of this peptide, before reaching floor levels and remaining relatively stable throughout the main part of the symptomatic phase [35].

Here, as expected, both sEOAD and PSEN1 groups had CSF $A\beta_{42}$ levels below standard cut-offs [17], and significantly lower CSF $A\beta_{42}$ levels compared with controls. However, PSEN1 also had significantly lower mean levels of $A\beta_{42}$ compared with sEOAD. To the best of our knowledge, this is the first report of a significant difference in CSF $A\beta_{42}$ levels in genetic as opposed to sporadic EOAD. Published data addressing this specific issue are limited. Ringman and colleagues described a *PSEN1* mutation (S212Y) in a subject with very low CSF $A\beta_{42}$ levels as compared with similarly impaired controls from the Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort [36]. Previous studies comparing $A\beta_{42}$ levels between sporadic and genetic AD used LOAD subjects and the mutation carriers were, on many occasions, pre-symptomatic [37, 38]. We do not believe that our finding can be attributed to the age difference between the groups, as $A\beta_{42}$ levels vary little with age in the normal population [17] and, in fact, our PSEN1 subjects were 10 years younger than the sporadic EOAD group. A further point to make is that carriers of 6 different *PSEN1* mutations were analyzed, and therefore this difference does not seem to be related to a specific mutation, although we do acknowledge the possibility that not all *PSEN1* mutations might exhibit this pattern.

The lower CSF $A\beta_{42}$ levels in PSEN1 probably reflect a greater $A\beta_{42}$ cerebral load in these subjects compared to sEOAD. In this context, neuropathological studies demonstrate that PSEN1 have a higher $A\beta_{42}$ percentage in plaques and a greater total cerebral load of $A\beta_{42}$, irrespective of mutation type, compared to sporadic AD, there being no reported correlation between the duration of clinical symptoms and total $A\beta_{42}$ load [9, 39–41].

A typical 'AD signature' consisting of cortical thinning encompassing the medial temporal lobe, lateral temporo-parietal regions and medial parietal regions such as the precuneus has already been described [42], even in mild clinical phases, and reflects the regions of maximum AD vulnerability. The present study replicates these findings: both PSEN1 and sEOAD presented widespread cortical atrophy encompassing typically affected AD areas. Amyloid deposition in PSEN1 starts in the striatum more than a decade before symptom onset [43] and maintains a different pattern of uptake, with higher striatal and somewhat lower cortical PIB retention in familial AD compared with sporadic AD, even in symptomatic phases; this is suggestive of possible differences in CTh loss pattern in the two groups [44, 45]. By contrast, our study revealed no significant differences between AD groups in relation to CTh maps. There was also a significant overlap between the two patterns of grey matter loss in cortical and subcortical structures measured with VBM (Fig. 4) and no difference survived after MMSE correction. Similar non-significant results in subcortical structures were confirmed with a post-hoc volume-based subcortical Free Surfer stream analysis. Likewise, we found no significant correlations between $A\beta_{42}$ and CTh, suggesting that the cortical loss in these patients is, at these stages, independent of the $A\beta_{42}$ load. We acknowledge that these non-significant results could be a biased result due to the small number of subjects in each group, especially in the PSEN1 group. However, previous larger studies in late-onset AD also demonstrate a lack of correlation between total brain amyloid load, as measured by PIB or $A\beta_{42}$ CSF values or amyloid distribution, and the degree or extension of cerebral atrophy or hypometabolism in subjects with AD dementia [5, 46–48]. In addition, in postmortem studies the rates of whole-brain atrophy or dementia severity were not correlated with the total Aβ plaque load in patients with late-onset AD dementia [49–51]. Taken together these data support the idea that, in the dementia stages of the disease, the effect of $A\beta_{42}$ cerebral load on cognition or brain structure is not direct.

Our results for the whole AD sample (PSEN1 and sEOAD) revealed a negative CTh/t-tau correlation in two left-hemisphere regions, the superior parietal and the caudal middle frontal cortex. This is consistent with previous research in late-onset subjects showing that, in the symptomatic phases of AD, the degree and extent of neuronal loss, with the consequent cognitive impairment and cortical atrophy, bear more relationship to tau levels and neurofibrillary pathology than to the amyloid load [46, 51]. In fact, even in cognitively preserved controls, high levels of CSF t-tau were associated with reduced grey matter volume and reduced metabolism in typically AD-targeted regions such as the precuneus and posterior cingulate, reflecting possible preclinical disease [52, 53]. The fact that we obtained a significant correlation in restricted areas in only one hemisphere may be due to the small sample size.

In contrast to the findings for $A\beta_{42}$, no differences between sEOAD and PSEN1 were observed with respect to t-tau or p-tau levels. It remains a matter of controversy whether t-tau and p-tau reflect neurofibrillary tangle load or neurodegeneration [46, 54]. Previous neuropathological studies have shown some discrepancies in the effect of PSEN1 mutations on the accumulation of neurofibrillary tangles and neuronal loss. Gregory and co-workers observed more hippocampal and cortical atrophy in genetic AD compared with sporadic late-onset AD, despite a similar disease duration, although the subjects did have very different ages at death [55]. Gomez-Isla et al. found that although all the *PSEN1* and *PSEN2* mutations they studied led to enhanced deposition of total Aβ and $A\beta_{42}$, only some of these mutations were also associated with faster rates of neurofibrillary tangle formation and neuronal loss [40]. Moreover, the same PSEN1 mutation can lead to a wide range of neurofibrillary burden, suggesting the interaction of genotype with specific individual factors [40].

We believe that the lack of differences between PSEN1 and sEOAD in t-tau and p-tau levels and grey matter loss are related phenomenon. Specifically, we interpret our findings as showing that PSEN1 and sEOAD have, at a stage of mild or moderate dementia, the same level of neurodegeneration, despite an increased $A\beta_{42}$ load in genetic AD. The similarity of CTh maps would, in addition, indicate that although the initial pathogenic mechanism may be different in the two groups they will finally converge, with the same structures being affected to a similar degree. Mutations in PSEN1 would, therefore, modify the age of disease onset but not the level or rate of neuronal injury.

The role of amyloid load in the natural history of AD and the link between amyloid load and neurodegeneration has been discussed in the literature [35, 54], with different theoretical scenarios being proposed. One suggests that the neurodegenerative process depends on the amyloid load throughout the disease course (i.e., Aβ deposition is continuously required for AD progression and there is a direct relationship between Aβ load and neurodegeneration). A second scenario is that neurodegeneration starts only when the amyloid load reaches a determined threshold level, and then becomes Aβ independent. Finally, there is the suggestion that amyloid deposits act as a trigger for neurodegeneration at the very beginning of the disease, after which the process becomes amyloid-independent [56].

Our findings support the third possibility. In this population no correlation was observed between grey matter loss and amyloid levels that would support the first option. Furthermore, if lower floor levels of CSF $A\beta_{42}$ support higher Aβ load in PSEN, as discussed above, then the critical threshold of Aβ would be reached earlier in the course of the disease in PSEN1; thus, a greater level of neurodegeneration (i.e., grey matter loss) should have been observed in PSEN1 if the second option were correct.

We acknowledge that the relatively low number of subjects is the main limitation of the present study, although this is an inherent limitation when dealing with monogenic AD. While it seems clear that reduced CSF $A\beta_{42}$ levels correlate with amyloid deposition we accept that, at present, there is no definitive evidence that CSF $A\beta_{42}$ levels reflect in a quantitative-dependent manner the extent of amyloid deposition. Furthermore, the issue of whether the CSF $A\beta_{42}$ levels reflect only fibrillar or both fibrillar and soluble amyloid species is not completely resolved, despite the consistent PIB correlations. All our subjects are presently living and we therefore have no neuropathological data of cerebral $A\beta_{42}$ load or neurofibrillary pathology in these individuals.

In conclusion, PSEN1 subjects, with a lifetime-increased $A\beta_{42}$ production, had lower $A\beta_{42}$ CSF levels, but equivalent t-tau and p-tau levels, compared with sEOAD, thereby suggesting a greater $A\beta_{42}$ cerebral load in these patients. These possible differences in $A\beta_{42}$ deposition were not significantly correlated with the degree of grey matter loss, and CTh was only correlated with t-tau. The lack of significant differences in t-tau and p-tau levels and in the severity of CTh or grey matter loss suggests a similar level of neuronal injury in spite of higher $A\beta_{42}$ load in PSEN1.

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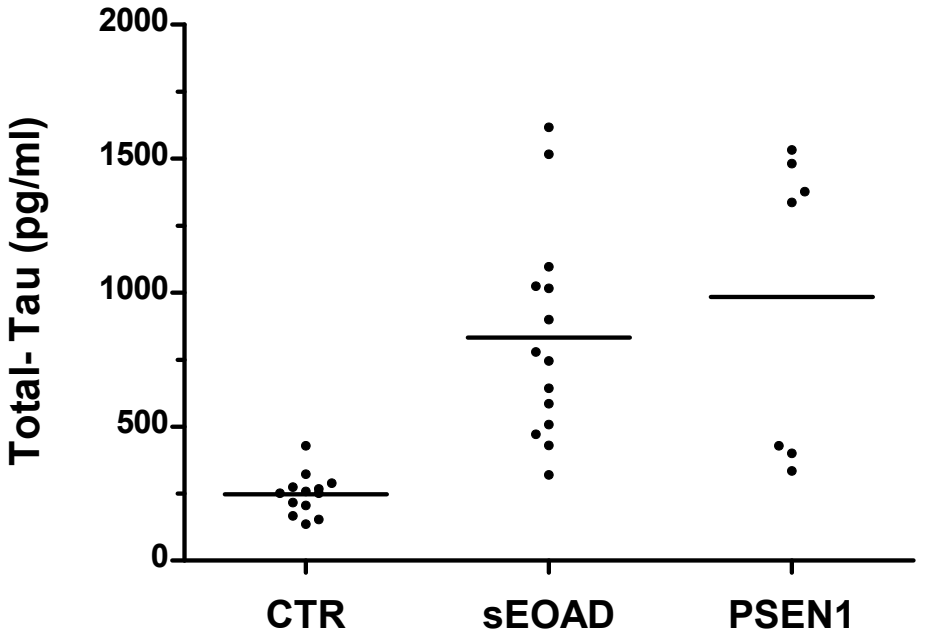
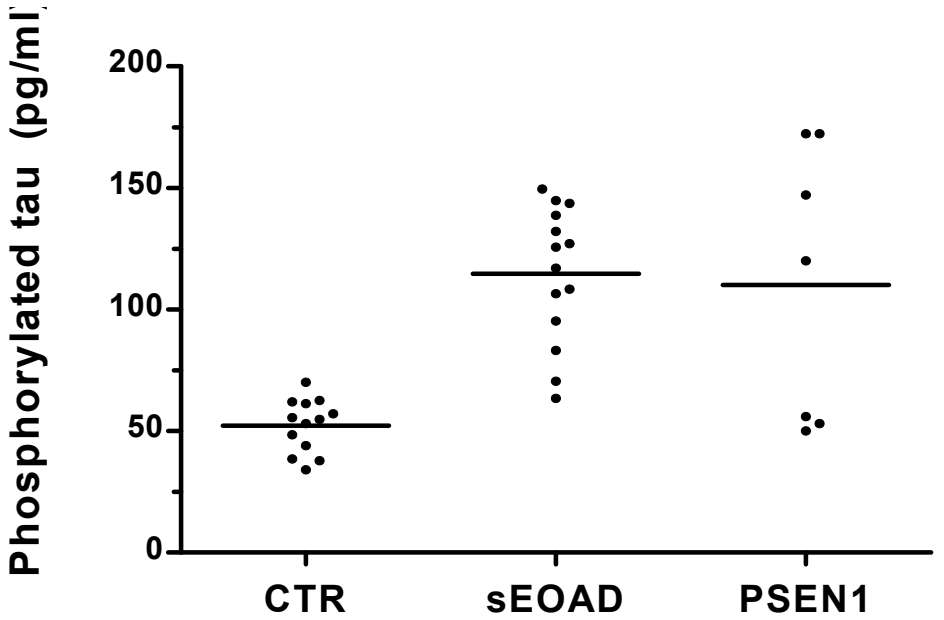
Supplementary Figure 1: Distribution of CSF t-tau values in the different study groups. PSEN1 = *PSEN1* mutation carriers; sEOAD = sporadic early-onset AD; CTR = controls



Supplementary Figure 2: Distribution of CSF p-tau values in the different study groups. PSEN1 = *PSEN1* mutation carriers; sEOAD = sporadic early-onset AD; CTR = controls



Supplementary Table 1: Mean group values and post-hoc comparisons between groups. EIV and subcortical values are volume estimations, whereas the cortical ROI values are expressing cortical thickness measures. sEOAD = sporadic early-onset AD; PSEN1 = carriers of *PSEN 1* mutations; CTR = controls

	CTR	sEOAD	PSEN1	CTR vs. sEOAD	CTR vs. PSEN1	sEOAD vs. PSEN1
EIV	1441426 (160518)	1408693 (180461)	1432314 (124750)	p=0.868	p=0.992	p=0.320
Left Superior Frontal	2.762 (.133)	2.620 (.111)	2.587 (.100)	p=0.013*	p=0.008*	p=0.820
Left Thalamus	6788 (830)	6311 (801)	6217 (597)	p=0.279	p=0.265	p=0.963
Left Hippocampus	4177 (598)	3299 (574)	3691 (562)	p=0.001*	p=0.185	p=0.327
Right Thalamus	7083 (865)	6307 (647)	6403 (606)	p=0.030*	p=0.128	p=0.958
Right Hippocampus	4280 (575)	3331 (636)	3723 (449)	p=0.001*	p=0.109	p=0.320

Supplementary Table 2:

Contrast	Voxels	t-value	X (MNI)	Y (MNI)	Z (MNI)	Brain region
CTR > sEOAD	37166	5.18	-4	-56	24	Precuneus / PCC
	390	4.02	8	-68	-28	Cerebellum
CTR > PSEN1	36842	6.20	-54	-30	-14	MTC
	150	4.05	-36	6	32	L MFG
	148	3.23	-28	4	44	L MFG
	143	3.82	-14	20	36	L PaC
	41	3.31	-16	48	26	L Frontal Pole
sEOAD > PSEN1	388	4.58	70	-12	4	R STG
	369	2.33	36	-4	-30	R Fusiform Gyrus
	335	3.12	10	-22	16	Right Thalamus
	262	2.44	-32	-26	10	L Insular Cortex
	260	3.38	10	36	36	R PaC
	208	2.50	-4	20	20	L ACC
	184	2.75	14	-64	38	R Precuneus
	181	2.68	56	-72	-16	R LOC
	80	2.22	-10	-58	42	L Precuneus
	51	1.79	56	-42	-30	R ITG

VBM results for the Controls vs. AD group contrasts. Coordinates are shown according to the Montreal Neurological Institute (MNI) atlas. Only clusters with at least 30 voxels are shown. SFG = superior frontal gyrus; MFG = middle frontal gyrus; PaC = paracingulate cortex; AAC = anterior cingulate cortex; STG = superior temporal gyrus; ITG = inferior temporal gyrus; LOC = lateral occipital cortex * Controls > sEOAD and Controls > PSEN1 contrasts are corrected by multiple comparisons and thresholded at $p<0.05$. sEOAD > PSEN1 is a $p<0.001$ uncorrected contrast.

Supplementary Table 3. Areas with significant values in cortical thickness comparisons (sEOAD vs. CTR, PSEN1 vs. CTR, CTh/t-tau correlation) after FWE corrections for multiple comparisons using Monte Carlo simulations (10000 permutations). Cortical areas corresponding to each cluster were assigned using the parcellations from an available atlas [12]

Comparison	Size (mm^2)	Max p value	Max-p MNI coordinates	Cluster-wise probability	Cluster-wise confidence interval	Atlas label
Total tau – CTh correlation	3083.23	$10^{(-2.83)}$	(-27.47, 49.94, 43.88)	0.0003	(0.0001-0.0050)	Superior parietal
	1497.01	$10^{(-2.47)}$	(-27.17, -4.89, 44.25)	0.0429	(0.0403-0.04550)	Caudal middle frontal
sEOAD < CTR Left Hemisphere CTh	30936.43		(-27.47, 50.39, 37.89)	0.0001	(0.0000-0.0002)	Inferior parietal
	2693.51		(-35.45, 1.30, 31.30)	0.0008	(0.0005-0.0012)	Caudal middle frontal
sEOAD < CTR Right Hemisphere CTh	29985.29		(42.9293 - 49.1, -12.25)	0.0001	(0.0000-0.0002)	Inferior temporal
	2443.39		(20.30, 25.46, 46.29)	0.0010	(0.0006-0.0014)	Superior frontal
PSEN1 < CTR Left Hemisphere CTh	35710.22		(-16.36, -71.72, 60.64)	0.0001	(0.0000-0.0002)	Superior parietal
PSEN1 < CTR Right Hemisphere CTh	33937.50		(44.14, -38.92, 2.29)	0.0001	(0.0000-0.0002)	Bank of superior temporal sulcus

Trabajo número 3

A preliminary study of the whole-genome expression profile of sporadic and monogenic early-onset Alzheimer's disease.

Antonell A, Lladó A, Altirriba J, Botta-Orfila T, Balasa M, Fernández M, Ferrer I,

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RESULTADOS

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A preliminary study of the whole-genome expression profile of sporadic and monogenic early-onset Alzheimer's disease

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ABSTRACT

Alzheimer's disease (AD) is the most common neurodegenerative dementia. Approximately 10% of cases present at an age of onset before 65 years old, which in turn can be monogenic familial AD (FAD) or sporadic early-onset AD (sEOAD). Mutations in *PSEN1, PSEN2*, and *APP* genes have been linked with FAD. The aim of our study is to describe the brain whole-genome RNA expression profile of the posterior cingulate area in sEOAD and FAD caused by *PSEN1* mutations (FAD-PSEN1). Fourteen patients (7 sEOAD and 7 FAD-PSEN1) and 7 neurologically healthy control subjects were selected and whole-genome expression was measured using Affymetrix Human Gene 1.1 microarrays. We identified statistically significant expression changes in sEOAD and FAD-PSEN1 brains with respect to control subjects (3183 and 3350 differentially expressed genes [DEG] respectively, false discovery rate-corrected $p < 0.05$). Of them, 1916 DEG were common between the 2 comparisons. We did not identify DEG between sEOAD and FAD-PSEN1. Microarray data were validated through real-time quantitative polymerase chain reaction. In silico analysis of DEG revealed an alteration in biological pathways related to intracellular signaling pathways (particularly calcium signaling), neuroactive ligand-receptor interactions, axon guidance, and long-term potentiation in both groups of patients. In conclusion, the altered biological final pathways in sEOAD and FAD-PSEN1 are mainly related with cell signaling cascades, synaptic plasticity, and learning and memory processes. We hypothesize that these 2 groups of early-onset AD with distinct etiologies and likely different could present a neurodegenerative process with potential different pathways that might converge in a common and similar final stage of the disease.

1. Introduction

Alzheimer's disease (AD) is the most frequent cause of neurodegenerative dementia, which leads to cognitive and behavioral impairments (McKhann et al., 1984, 2011). According to the age of onset of symptoms, AD can be arbitrarily divided in 2 groups: late-onset AD (LOAD) (onset ≥65 years old) (approximately 90% of cases) and early-onset AD (EOAD) (onset <65 years old) (approximately 10% of cases) (Koedam et al., 2010; van der Flier et al., 2011). Genetically, AD can be classified in 2 forms: (1) familial or monogenic forms of AD (FAD) (0.1%–0.5%), with an autosomal dominant pattern of inheritance, and early-onset AD caused by rare and highly penetrant mutations in 3 genes: the amyloid precursor protein gene (*APP*), the presenilin 1 gene (*PSEN1*), and the presenilin 2 gene (*PSEN2*); and (2) polygenic and/or multifactorial or so-called "sporadic" cases with less apparent or no familial aggregation and usually of later age at onset. After hundreds of association genetic studies and genome-wide association studies of thousands of patients with LOAD, different genes have been identified as risk factors, (http://www.alzgene.org/) (Bertram et al., 2007), although only the presence of the allele ε4 of the apolipoprotein E gene (*APOE*) has been unequivocally established as a genetic risk factor for AD.

AD can be clinically heterogeneous and the frequency of atypical clinical presentations is different between EOAD, FAD, and LOAD (Balasa et al., 2011; Gomez-Tortosa et al., 2010). However, the underlying neuropathological findings between them are similar, with extracellular deposition of amyloid-β peptide plaques, intracellular neurofibrillary tangles—that consist of hyperphosphorylated aggregates of the microtubule-associated protein tau—and selective neuronal loss (Braak and Braak, 1997).

Microarray technology allows the measurement of the expression of many thousands of genes simultaneously. There are multiple array-based choices for surveying genome-wide gene expression

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that differ in content, probe preparation methods, and chemistry of the array. During the past decade many studies have investigated human gene expression changes in AD, mainly in brain tissue (Avramopoulos et al., 2011; Blalock et al., 2004, 2011; Booij et al., 2011; Colangelo et al., 2002; Dunckley et al., 2006; Emilsson et al., 2006; Ginsberg et al., 2000; Grunblatt et al., 2007; Haroutunian et al., 2009; Katsel et al., 2009; Liang et al., 2008, 2010; Loring et al., 2001; Lukiw and Crapper McLachlan, 1990; Parachikova et al., 2007; Pasinetti, 2001; Podtelezhnikov et al., 2011; Ray et al., 2008; Ricciarelli et al., 2004; Yao et al., 2003). Data obtained until now have yielded important new insights into the possible disease mechanisms and consequences of the disease at the molecular level. Multiple processes have been implicated in AD, notably including biological pathways related to synaptic function and neurotransmission, signal transduction, energy metabolism, oxidative stress, calcium signaling, and cytoskeleton protein processing or misfolding, inflammation, and cholesterol synthesis (Blalock et al., 2004; Emilsson et al., 2006; Simpson et al., 2011). Nevertheless, all of these studies have been focused in sporadic LOAD, and to our best knowledge, there are no studies on sporadic EOAD (sEOAD) or FAD.

The aim of this study is to define the brain differential gene expression profile in 2 different groups of EOAD patients, sEOAD and genetically determined FAD caused by *PSEN1* mutations (FAD-PSEN1), with respect to control subjects. We also wanted to find possible differences between them to infer if distinct pathological cascades are implicated in the pathogenesis of sEOAD and FAD.

2. Methods

2.1. Subjects

All brain samples were provided by the Neurological Tissue Bank of the Biobank-Hospital Clínic-IDIBAPS and the Neuropathology Institute from the Hospital Universitari de Bellvitge. Neuropathological examination was performed according to standardized protocols. Disease evaluation and classification was performed according to international consensus criteria (Hyman et al., 2012; Montine et al., 2012).

Isolation of genomic DNA from brains for mutational screening and *APOE* genotyping was carried out using the QIAamp DNA Minikit (Qiagen, Valencia, CA, USA) following manufacturer's instructions. *APOE* genotype was determined using polymerase chain reaction (PCR) amplification and *Hha*I restriction enzyme in all subjects. We selected 14 cases that fulfilled neuropathological criteria of AD. Seven of them were already diagnosed as carriers of a mutation in *PSEN1* gene (4 subjects with the M139T mutation, 2 with the V89L mutation, and 1 with the E120G mutation). The remaining 7 cases were screened for mutations in the *PSEN1*, *PSEN2*, and *APP* genes as previously described (Antonell et al., 2012), but no mutation was detected. Family history of the disease was negative in 5 cases and it was not available in 2 cases. We cannot completely rule out the presence of a mutation in a yet unknown gene causative of monogenic AD different from *PSEN1*, *PSEN2*, and *APP* genes in these 2 cases with nonavailable family history. Furthermore, 7 brain samples of control subjects without signs of neurodegenerative disease in the neuropathological study were included. The 3 groups were matched for sex, postmortem delay (PMD), and *APOE* genotype. Control subjects and FAD-PSEN1 groups were also matched for age, but age in sEOAD subjects presented statistically significant differences (analysis of variance with a Tukey post hoc test) when compared with the other 2 groups: FAD-PSEN1 ($p < 0.01$) and control subjects ($p < 0.001$). Their demographic and neuropathological characteristics are shown in Table 1. All participants or their legal representatives provided written informed consent for the study, which was approved by the Ethics Committee of the Hospital Clínic of Barcelona.

Table 1
Characteristics of the subjects included in this study and their classification in 3 groups

Subject	Sex	Age (y)	*APOE* genotype	RIN value	PMD (h:min)	AD neuropathologic change[a]	Group
E1	Female	63	3/3	7	9:00	A3, B3, C3, and CAA moderate	sEOAD
E2	Female	65	3/3	6	16:00	A3, B3, C3	sEOAD
E3	Male	57	4/3	6.8	4:30	A3, B3, C3, and CAA severe	sEOAD
E4	Male	69	3/3	8.5	3:30	A3, B3, C3, and CAA moderate	sEOAD
E5	Male	61	3/3	7.3	19:15	A3, B3, C3, and CAA mild	sEOAD
E6	Male	68	3/3	7.1	9:00	A3, B3, C3, and CAA severe	sEOAD
E7	Male	60	3/3	8.4	5:00	A3, B3, C3, and CAA moderate	sEOAD
		63 (1.6)		7.3 (0.3)	09:30 (2.3)		
P1	Female	48	4/3	6.8	16:25	A3, B3, C3, and CAA severe	FAD-PSEN1 (M139T)
P2	Male	53	3/3	7.9	5:15	A3, B3, C3, and LBD limbic predominant	FAD-PSEN1 (M139T)
P3	Male	54	3/3	7.9	7:30	A3, B3, C3, and CAA moderate	FAD-PSEN1 (V89L)
P4	Male	64	3/3	6.0	14:45	A3, B3, C3, and CAA moderate	FAD-PSEN1 (M139T)
P5	Male	57	3/3	7.4	15:15	A3, B3, C3, and CAA severe	FAD-PSEN1 (M139T)
P6	Male	57	3/2	6.7	9:30	A3, B3, C3, and CAA severe	FAD-PSEN1 (V89L)
P7	Male	44	3/3	8.3	5:30	A3, B3, C3, and CAA severe	FAD-PSEN1 (E120G)
		54 (2.5)		7.3 (0.3)	10:36 (1.8)		
C1	Female	45	3/3	6.1	14:40	No neuropathological findings	Control
C2	Female	50	3/3	7.2	12:00	No neuropathological findings	Control
C3	Male	58	4/3	8.0	4:00	No neuropathological findings	Control
C4	Female	46	3/2	7.5	9:35	No neuropathological findings	Control
C5	Male	47	3/3	6.4	4:55	No neuropathological findings	Control
C6	Male	49	3/3	7.1	7:35	No neuropathological findings	Control
C7	Male	53	3/3	7.9	7:25	No neuropathological findings	Control
		50 (1.7)		7.2 (0.3)	08:36 (1.4)		

For each group, mean (standard error of the mean) of age, RIN values, and PMD are shown.
Key: AD, Alzheimer's disease; CAA, cerebral amyloid angiopathy; CERAD, Consortium to Establish a Registry for Alzheimer's Disease; FAD-PSEN1, familial Alzheimer's disease caused by a mutation in the *PSEN1* gene; LBD, Lewy body disease; PMD, postmortem delay; RIN, RNA integrity number; sEOAD, sporadic early-onset Alzheimer's disease.
[a] Alzheimer's disease neuropathologic change according to the new National Institute of Aging/Alzheimer's Association guidelines 2012. "ABC" score should be read as follows.
"A" for Thal phase for Abeta plaques: 0 = none, 1 = phase 1 or 2, 2 = phase 3, and 3 = phase 4 or 5.
"B" for Braak and Braak neurofibrillary stage: 0 = none, 1 = stage I or II, 2 = stage III or IV, and 3 = stage V or VI.
"C" for CERAD neuritic plaque score: 0 = none, 1 = sparse, 2 = moderate, and 3 = frequent.

2.2. RNA and cDNA preparations

Isolation of total RNA from frozen brain tissue of the posterior cingulate area at the thalamus level was performed using RNeasy Lipid Tissue Mini Kit (Qiagen). This brain area was chosen because it is already affected in limbic stages of AD and it is metabolically affected (by hypometabolism) in the preclinical stages (Braak et al., 2006; Mevel et al., 2007). The quality of all RNA samples was checked using an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA). All samples used for hybridization had an RNA Integrity Number from 6 to 8.5 (Table 1). Total RNA (1.2 μg) was converted to cDNA using SuperScript III primed with random primers, with the SuperScript VILO Mastermix (Invitrogen, Paisley, UK).

2.3. Microarray hybridization

Gene expression was assessed using the GeneChip Human Gene 1.1 ST Array Plate Affymetrix, which interrogates more than 28,000 well-annotated genes (human genome sequence assembly UCSC hg18, NCBI Build 36) with more than 750,000 distinct probes (Affymetrix, Santa Clara, CA, USA). Total RNA from patients and control subjects was labeled and converted to single-stranded DNA (ssDNA) according to the standard Affymetrix Terminal Labeling Kit protocol. After fragmentation, 5 μg of ssDNA was hybridized for 16 hours at 45 °C following Affymetrix standard procedures. GeneChips were scanned using a GeneTitan Affymetrix instrument.

2.4. Accession numbers

Data discussed in this publication have been deposited in the NCBI Gene Expression Omnibus (Barrett et al., 2011; Edgar et al., 2002) and are accessible through the GEO Series accession number GSE39420.

2.5. Bioinformatic and statistical nalysis

Robust Multi-array Analysis (Irizarry et al., 2003) was performed for background adjustment, normalization, and data summarization using the Affy software (version 1.30.0) (Gautier et al., 2004) from Bioconductor (Gentleman et al., 2004) in R language (R Development Core Team, 2011; http://www.r-project.org) (version 2.13.2). To increase the sensitivity of the analysis and reduce background noise, genes that presented an intensity value lower than the 50th percentile of all values in 4 or more samples in all of the groups were removed. Processed, adjusted, and filtered data were analyzed using the LIMMA software (version 3.8.2) (Smyth, 2004). Pairwise comparisons were performed among the 3 groups of subjects (control, sEOAD, and FAD-PSEN1). The selection of differentially expressed genes (DEG) between conditions was based on a linear model with empirical Bayes moderation of the variance estimates following the method developed by Smyth (2004). The associated p values of the moderated t statistic were adjusted for multiple testing with the Benjamini and Hochberg method to keep the false discovery rate (Huang da et al., 2009) less than 5%. Genes with adjusted p values less than 0.05 were considered DEG.

2.6. Real-time quantitative PCR and data analysis

Real-time quantitative PCR (RT-qPCR) was performed on a selected set of genes (all DEG) to validate microarray data. Primers and probes were designed using the ProbeFinder software version 2.45 from Roche, with default parameters. All primers were designed to span an intron and are shown in Supplementary Table 1. Two reference genes were selected on the basis of their stability in expression levels in brain tissue (Leduc et al., 2011; Penna et al., 2011): *TOP1* and *GPS1*. PCR reactions were set up in a 10-μL volume in a 384-well plate with 3 replicates per sample. SYBR green master mix (Applied Biosystems, Carlsbad, CA) was used. RT-qPCRs were run in a VIIA 7 Real-Time PCR System (Applied Biosystems) with the following conditions: 50 °C for 2 minutes, 95 °C for 10 minutes, and 40 cycles of 95 °C for 15 seconds and 60 °C for 1 minute. A final melting curve was performed to ensure that only 1 amplicon was present. Raw data were obtained using VIIA 7 v1.0 software (Applied Biosystems). Subsequent measurements of relative expression were carried out using Excel (Microsoft Office Excel 2003), with the standard curve method. Patients' expression values were normalized with respect to expression values obtained from control subjects and represented in base 2 logarithm to obtain a continuous and symmetric scale. Differences in gene expression between patients and control subjects were determined by analysis of variance with a Tukey post hoc test. The correlation between the data obtained by the microarrays and the RT-qPCR was calculated using the Spearman rank correlation coefficient (ρ) and the associated p value. Both tests were performed using GraphPad Prism software (version 5.04).

2.7. Pathway definition

To restrict the selection criteria of genes submitted to analysis we examined DEG that differed by a minimum of a 2-fold change between groups. To obtain the Kyoto Encyclopedia of Genes and Genomes biological pathways affected in each comparison, we explored information obtained from 3 databases: Onto-Tools Pathway-Express (http://vortex.cs.wayne.edu/projects.htm#Onto-Express) (Khatri et al., 2002, 2007), DAVID Bioinformatics Resources 6.7 (http://david.abcc.ncifcrf.gov/tools.jsp) (Huang da et al., 2009), and ConsensusPathDB Release 22 (http://cpdb.molgen.mpg.de/) (Kamburov et al., 2009, 2011; Pentchev et al., 2010).

3. Results

3.1. Whole genome expression profiling

To increase the sensitivity of the analysis and reduce background noise, probes were filtered according to their expression, and 17,767 probes were considered for further analysis. Among them, DEG were selected if they had an adjusted $p < 0.05$; when compared between control subjects and FAD-PSEN1 patients, we obtained 3350 DEG (1529 upregulated and 1821 downregulated), when compared between control subjects and sEOAD patients, we obtained 3183 DEG (1473 upregulated and 1710 downregulated), and when compared between FAD-PSEN1 and sEOAD patients, we obtained 0 genes. The complete list of the DEG is provided in the Supplementary data (Supplementary Table 2 and Supplementary Table 3). An important overlap between expression changes in sEOAD and FAD-PSEN1 patients compared with control subjects was found (1916 DEG were common).

3.2. Bioinformatic pathway definition and validation

To restrict the biological pathways analysis to genes with a higher biological relevance, the DEG were filtered and only those with a minimum 2-fold change versus the control subjects were analyzed. Therefore, we submitted a list of 1111 DEG (209 upregulated and 902 downregulated) for the comparison of control subjects with FAD-PSEN1 patients and, a list of 1042 genes (331 upregulated and 711 downregulated) for comparison of control

Table 2
Biological process categories (KEGG pathways) significantly overrepresented with respect to control subjects

Term	*p*	FDR	Genes
(A) Sporadic early-onset Alzheimer's disease			
Calcium signaling pathway	1.52E-13	1.78E-10	*ADCY1*, *DRD5*, *PPP3R1*, *ITPKA*, *ATP2B1*, *ATP2B2*, *PDE1B*, *PTK2B*, *PDE1A*, *CAMK2B*, *CAMK2A*, *HTR5A*, *SLC8A1*, *SLC8A2*, *CCKBR*, *SPHK1*, *CACNA1I*, *GRIN1*, *GRIN2A*, *PRKCG*, *GRM1*, *PRKCB*, *GRM5*, *PLCE1*, *CAMK4*, *CHRM3*, *CHRM1*, *ADRA1B*, *CACNA1G*, *CALM3*, *RYR2*, *CACNA1E*, *CACNA1C*, *CACNA1D*, *ADRA1D*, *CACNA1A*, *CACNA1B*, *HTR2A*
Neuroactive ligand-receptor interaction	3.95E-09	4.65E-06	*GRIK1*, *GABRB3*, *GABRB2*, *GRIK2*, *GRIK3*, *DRD5*, *OPRK1*, *GLRA3*, *LPAR4*, *GABBR2*, *VIPR1*, *HTR1A*, *HRH3*, *GRIN2B*, *HTR5A*, *GABRQ*, *GABRD*, *GABRG2*, *GLRB*, *C5AR1*, *GABRA1*, *CCKBR*, *GABRA4*, *GABRA3*, *GABRA5*, *GRIN1*, *GRIN2A*, *GRM1*, *GRM5*, *GRM4*, *GRM2*, *CHRM3*, *GRIA2*, *SSTR1*, *CHRM1*, *GRM7*, *ADRA1B*, *ADRA1D*, *HTR2A*
MAPK signaling pathway	4.31E-08	5.07E-05	*IL1R2*, *FGF9*, *FGF14*, *MKNK2*, *PPP3R1*, *CACNB2*, *CACNB3*, *FGF13*, *FGF12*, *NFKB2*, *CACNB4*, *GNG12*, *TGFB2*, *HSPA1L*, *FOS*, *RASGRP1*, *HSPA6*, *HSPA7*, *PAK1*, *PTPN5*, *CACNA1I*, *PTPRR*, *NR4A1*, *PRKCG*, *CACNG3*, *CACNG2*, *FLNC*, *CACNA2D3*, *CACNA2D2*, *PRKCB*, *RPS6KA6*, *DUSP1*, *CACNA1G*, *HSPB1*, *CACNA1E*, *CACNA1C*, *CACNA1D*, *CACNA1A*, *CACNA1B*
Long-term potentiation	1.86E-06	2.19E-03	*ADCY1*, *GRIN1*, *GRIN2A*, *PPP3R1*, *PRKCG*, *GRM1*, *PRKCB*, *GRM5*, *RPS6KA6*, *CAMK4*, *GRIA2*, *GRIN2B*, *CALM3*, *CAMK2B*, *CACNA1C*, *CAMK2A*
Axon guidance	1.24E-05	1.46E-02	*ABLIM2*, *LIMK1*, *PPP3R1*, *L1CAM*, *CDK5*, *SLIT1*, *SLIT3*, *EPHA5*, *PAK6*, *PAK7*, *EPHA4*, *SEMA6B*, *EPHA6*, *CXCR4*, *UNC5A*, *PAK3*, *SEMA3E*, *ROBO2*, *UNC5D*, *PAK1*, *SEMA4A*
(B) Familial Alzheimer's disease caused by a mutation in the *PSEN1* gene			
Calcium signaling pathway	1.29E-12	1.52E-09	*ADCY1*, *DRD5*, *PPP3R1*, *ITPKA*, *ATP2B1*, *ATP2B2*, *ATP2B3*, *PDE1B*, *PTK2B*, *CAMK2B*, *PLCB1*, *CAMK2A*, *HTR5A*, *SLC8A1*, *SLC8A2*, *CCKBR*, *CACNA1I*, *GRIN1*, *HTR4*, *PRKCG*, *GRM1*, *ITPR1*, *PRKCB*, *GRM5*, *P2RX5*, *GNAL*, *CD38*, *PLCE1*, *CAMK4*, *CHRM3*, *ADRA1B*, *CACNA1G*, *CALM3*, *CACNA1E*, *CACNA1C*, *ADRA1D*, *CACNA1A*, *CACNA1B*, *HTR2A*
Neuroactive ligand-receptor interaction	7.31E-11	8.61E-08	*GPR83*, *C3AR1*, *MCHR2*, *GABRB3*, *GRIK1*, *THRB*, *GABRB2*, *GRIK2*, *DRD5*, *OPRK1*, *GLRA3*, *LPAR4*, *GABBR2*, *VIPR1*, *APLNR*, *HTR1A*, *HRH3*, *GRIN2B*, *HTR5A*, *GABRQ*, *HTR1E*, *GABRD*, *GABRG2*, *GLRB*, *GABRG3*, *GABRA1*, *CCKBR*, *GABRA4*, *RXFP1*, *GABRA3*, *GABRA5*, *GRIN1*, *HTR4*, *GRM1*, *GRM5*, *P2RX5*, *GRM4*, *SSTR2*, *GRM2*, *CHRM3*, *SSTR1*, *GRM7*, *ADRA1B*, *ADRA1D*, *HTR2A*
Type 1 diabetes mellitus	5.30E-07	6.25E-04	*ICA1*, *HLA-DRB3*, *PTPRN2*, *HLA-DMB*, *PTPRN*, *HLA-DMA*, *HLA-DQA1*, *GAD2*, *HLA-DRB4*, *HLA-DPA1*, *HLA-DPB1*, *HLA-DOA*, *GAD1*, *HLA-DRA*
Axon guidance	1.72E-06	2.03E-03	*ABLIM2*, *EFNB3*, *LIMK1*, *PLXNA2*, *EFNA3*, *PPP3R1*, *L1CAM*, *CDK5*, *SLIT1*, *SLIT3*, *EPHA5*, *PAK6*, *PAK7*, *EPHA4*, *EPHB6*, *SEMA6B*, *EPHA7*, *CXCR4*, *UNC5A*, *PAK3*, *UNC5D*, *EFNA5*, *PAK1*, *SEMA4A*
Phosphatidylinositol signaling system	5.30E-06	6.25E-03	*SYNJ1*, *PI4KA*, *PIP5K1C*, *DGKH*, *PRKCG*, *DGKI*, *CDS1*, *ITPKA*, *ITPR1*, *PRKCB*, *PLCE1*, *DGKB*, *DGKE*, *INPP5J*, *CALM3*, *DGKZ*, *PLCB1*
Long-term potentiation	8.09E-06	9.53E-03	*ADCY1*, *GRIN1*, *PPP3R1*, *PRKCG*, *GRM1*, *ITPR1*, *PRKCB*, *GRM5*, *RPS6KA6*, *CAMK4*, *GRIN2B*, *CALM3*, *CAMK2B*, *CACNA1C*, *PLCB1*, *CAMK2A*

Significance was determined by a FDR corrected p value < 0.05, calculated using the DAVID Bioinformatics Resources database. Differentially expressed genes from these biological categories are listed.
Key: FDR, false discovery rate; KEGG, Kyoto Encyclopedia of Genes and Genomes; MAPK, mitogen-activated protein kinases.

subjects versus sEOAD patients. The altered biological pathways yielded by the analysis were very similar when they were assessed with the 3 different databases already mentioned. We found calcium signaling, neuroactive ligand-receptor interaction, axon guidance, and long-term potentiation, among others. It is worth noting that most of the genes in these pathways were downregulated. Despite some minor differences there was a high concordance between the 2 lists. Kyoto Encyclopedia of Genes and Genomes biological pathways assessed by the DAVID Bioinformatics Resources database are shown in Table 2 for each of the comparisons.

3.3. RT-qPCR validation of selected genes

To validate gene expression changes in the posterior cingulate brain region we performed RT-qPCR. Genes were selected for validation based on statistical significance ($p < 0.05$, corrected) and their key role in biological or signaling pathways altered in patients with respect to control subjects. Based on these criteria, 17 genes were evaluated: *ADCY*, *CACNA1B*, *CALM3*, *CAMK2A*, *CDK5*, *FOS*, *GABRA1*, *GABRG2*, *GAD2*, *GRIN2B*, *GRM1*, *LIMK1*, *PAK1*, *PIP5K1C*, *PLCE1*, *PRKCG*, and *STX1A*. Of them, 15 genes were downregulated and 2 genes were upregulated. Of the genes, 100% were validated for sEOAD patients and 94.1% were validated for FAD-PSEN1 patients. Overall, we have validated array data both in the direction and the magnitude of the change, with a high correlation between microarray and RT-qPCR data. Results from RT-qPCR analysis and correlation between RT-qPCR and microarray data are shown in Fig. 1 and Supplementary Table 4.

4. Discussion

We have used the microarray technique to screen the entire genome content for their expression alterations in the posterior cingulate brain region of sEOAD and FAD-PSEN1. We have found a list of >3000 DEG between each group of patients and control subjects. Some of these DEG were commonly deregulated in both groups of patients, and in the biological significance of the 2 lists of DEG there were also similarities. We therefore hypothesize that these 2 groups of EOAD patients with distinct etiologies and likely different could present a neurodegenerative process with potentially different pathways that might converge in a common and similar final stage of the disease. It is worth noting that cell types other than neurons might participate in many of the identified pathways.

We have found an alteration of several signal transduction pathways, such as calcium, mitogen-activated protein kinases, phosphatidylinositol, and insulin signaling. It is worth mentioning that the most significantly altered pathway in both groups of patients with respect to control subjects was the calcium signaling pathway. Deregulation of intracellular calcium signaling (increase in intracellular calcium) has already been implicated in the pathogenesis of AD. Calcium modulates many neural processes, including synaptic plasticity and apoptosis. An increase in amyloid metabolism or accumulation of amyloid-β (resulting from overproduction, altered processing, or a failure of clearance mechanisms) can lead to a remodeling of the calcium signaling system, which in turn can upset the delicate balance between the induction of long-term potentiation (LTP; learning mechanisms) and long-term depression

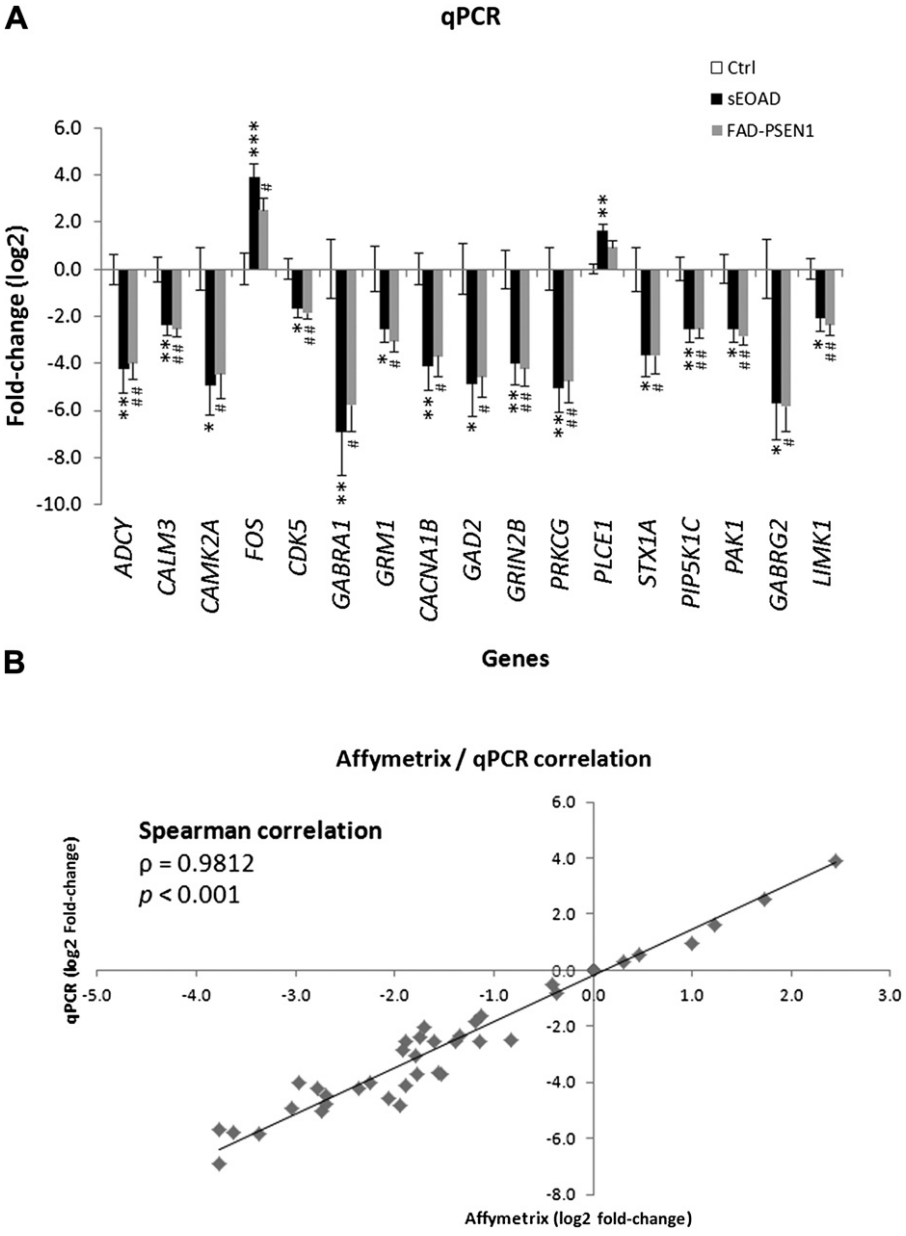

Fig. 1. Real-time qPCR validation of the microarray data. (A) Genes selected for validation by RT-qPCR are represented as the fold-change in log2 scale ($n = 7$; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ for Ctrl vs. sEOAD; # $p < 0.05$, ## $p < 0.01$ for Ctrl vs. FAD-PSEN1). (B) Correlation of the microarray and the qPCR data in fold-change log2 scale, with the Spearman rank correlation coefficient (ρ) and the associated p value. Abbreviations: Ctrl, control subjects; FAD-PSEN1, patients with familial Alzheimer's disease caused by a mutation in the *PSEN1* gene; log2, base 2 logarithm; qPCR, quantitative polymerase chain reaction; RT, real-time; sEOAD, sporadic early-onset Alzheimer's disease.

(loss of memory) leading to severe memory loss. Alternatively, calcium signaling alteration can lead to an alteration of amyloid metabolism (Berridge, 2010). There is also considerable evidence from transgenic mice, neural and nonneural cell lines, or primary neuron models that various AD mutations can induce changes in calcium signaling, as we have found in FAD-PSEN1 patients (LaFerla, 2002; Stutzmann, 2007).

The other biological pathways altered in our groups of patients are related to formation of neuronal network (axon guidance pathway) and neuronal function. We have found alteration of several synapses (GABAergic, glutamatergic, dopaminergic, and cholinergic), LTP, and neuroactive ligand-receptor interaction.

A prevailing view of AD pathogenesis is that synaptic dysfunction precedes neurodegeneration. In particular, defects in presynaptic neurotransmitter release could represent a converging early pathogenic event leading to neurodegeneration (Proctor et al., 2011). This hypothesis was prompted by genetic studies in adult mice in which loss of presenilin function resulted in progressive synaptic and memory impairments before age-dependent neurodegeneration. For instance, loss of presynaptic presenilin impairs glutamatergic neurotransmitter release by modulation of intracellular calcium release in presynaptic terminals and LTP, which is thought to be the cellular substrate of memory (Ho and Shen, 2011; Zhang et al., 2009).

Functional categories of genes identified as being differentially expressed in LOAD are numerous and varied, but some common themes arise: notably, intracellular signaling pathways—particularly calcium signaling—and neuroinflammation (Cooper-Knock et al., 2012). Interestingly, we have also found an alteration of calcium signaling (Emilsson et al., 2006) and regulation of genes

involved in neuronal functions (Avramopoulos et al., 2011; Yao et al., 2003) in EOAD.

Moreover, because aging is the major risk factor for the most common LOAD cases it is not surprising that there exists an overlap between the biological processes of normal aging and susceptibility to AD, suggesting that age-related gene expression changes might increase the risk of developing AD. In fact, it seems that transcriptional changes associated with aging and calcium homeostasis in the human brain are accelerated in patients with AD (Avramopoulos et al., 2011; Saetre et al., 2011).

We are aware that this study has limitations. The most important thing to be considered is RNA quality and PMD, which are not ideal and could affect the results of transcriptome analysis. In that sense, long PMDs do not allow the identification of changes in expression of messenger RNAs with a short half-life. However, PMDs of our samples were in the same range as in other studies with human brain samples, and the 3 groups that we compared present similar RNA Integrity Number and PMD range values.

The statistically significant difference in the ages between the sEOAD patients and the control subjects and FAD-PSEN1 patients is a potential confound because we worked with a reduced sample size. Although we detected gene expression differences between control subjects and both patient groups when we performed an accurate statistical analysis, the results of our array experiments should be interpreted with caution and should be validated in an independent and larger cohort. Moreover, patients analyzed in this work presented an advanced clinical (dementia) and pathological phase of the disease and this could be a reason for not observing any DEG between both groups of patients. On the other hand, because this study generated a large quantity of data, we restricted the number of genes submitted to overrepresentation analysis to genes that differed by a minimum of 2-fold between sample groups, which might have led to the loss of valuable information because genes with more moderate changes in expression were excluded.

Although we used patients in the final stages of the disease, it seems that biological pathways that we found altered in patients with respect to control subjects are among the earliest abnormalities that occur in AD, such as subtle alterations in neurotransmitter and neurotrophic factor signaling, perturbed cellular calcium homeostasis, and learning and/or memory processes (Berridge, 2010; Blalock et al., 2004, 2011; Gleichmann and Mattson, 2010; Liang et al., 2010; Proctor et al., 2011). It could indicate that these alterations probably occur at the beginning and remain until the final stages of the disease.

However, independently of the moment that gene expression changes occur, knowledge of gene expression changes involved in a disease might be useful in understanding the disease process and possibly exploring preventions and treatments, although the changes might not reflect the cause, but the effect of the disease. We identified common statistically significant expression changes in brains of patients with sEOAD and FAD-PSEN1 that might establish a degenerative link between the 2 cohorts. Our findings support a key role of calcium signaling and neuronal function in the pathogenesis of AD and suggest that patients with FAD could be of great utility and relevance to study the underlying mechanisms of AD. In that sense, future studies characterizing the gene expression and protein expression levels in living patients with AD at different clinical stages of the disease might be of value to understand the implication and role of the involved biological pathways.

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Disclosure statement

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All participants or their legal representatives provided written informed consent for the study, which was approved by the Ethics Committee of the Hospital Clínic of Barcelona.

Appendix A. Supplementary data

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Trabajo número 4

- en preparación –

Usefulness of CSF biomarkers in the differential diagnosis and prognosis of early-onset cognitive impairment

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RESULTADOS

Usefulness of CSF biomarkers in the differential diagnosis and prognosis of early-onset cognitive impairment

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Background: Both differential and early diagnosis as well as prognosis of early-onset cognitive impairment (< 65 years) are challenging due to the overlapping of symptoms of different neurodegenerative and non-degenerative disorders.

Objectives: To evaluate the usefulness of CSF Alzheimer's disease (AD) biomarkers (Aβ42, total-tau, phosphorylated-tau, Aβ42/p-tau ratio) in early-onset cognitive impairment differential diagnosis in clinical practice. To evaluate the capacity of CSF-AD biomarkers to predict subsequent decline in early-onset mild cognitive impairment (MCI).

Methods: Prospective recruitment of *de novo* subjects with early-onset cognitive impairment (< 65 years) (January 2009-March 2013). All participants underwent clinical and neuropsychological assessments, blood analysis, neuroimaging and a spinal tap. Initial clinical classification was based on criteria available in 2009. At the end of the study, all subjects were also prospectively reclassified according to the revised 2011 criteria for MCI, AD, frontotemporal dementia (FTD) and primary progressive aphasia, excluding CSF data. We compared CSF results between groups and evaluated the effect of adding the CSF results to the core clinical criteria of current criteria sets.

Results: 120 patients (51 MCI, 42 AD, 10 FTD, 3 posterior cortical atrophy and 14 primary progressive aphasia) and 37 controls were included. A threshold of 6.7 for the Aβ42/p-tau ratio best differentiated AD patients from controls; with 92% sensitivity, 94.6% specificity and AUC of 0.944. A total of 97% of the patients who met the revised NIA-AA core clinical criteria for AD presented pathological CSF values at inclusion; while only 4% of FTLD patients had such a profile. There were no differences in CSF biomarker levels between amnestic and non-amnestic presentations of AD. Inclusion of CSF results increased the probability of detection of the AD pathophysiological process to a high likelihood in 90% of amnestic presentation patients and 82% of the non-amnestic presentations. All the 24 MCI patients who progressed to AD dementia on follow-up presented pathological CSF levels at

baseline. Twenty-six out of the 27 MCI who remained stable or improved, showed normal baseline CSF-AD biomarkers.

Conclusions: CSF biomarkers, especially the Aβ42/p-tau ratio, provide high diagnostic accuracy in a clinical context in differentiating AD, FTLD and controls in presenile subjects and can be used equally in amnestic and non-amnestic presentations of AD to determine an increased likelihood of AD pathology. Abnormal CSF-AD biomarker levels predict subsequent progression to AD dementia in subjects with early-onset MCI.

Keywords: early-onset Alzheimer's disease, CSF biomarkers, Aβ42/p-tau ratio

Introduction

Alzheimer's disease (AD) has traditionally been conceptualized as a clinicopathological condition that requires the presence of the characteristic pathology hallmarks together with clinical dementia for its definitive diagnosis. Early-onset AD patients (EOAD), with clinical onset prior to 65 years of age, have a higher frequency of non-amnestic presentations (Stopford et al., 2008; Koedam et al., 2010; Smits et al., 2012) and greater diagnostic delays (Shinagawa et al., 2007; Koedam et al., 2010) than late-onset AD patients. The atypical clinical features of EOAD frequently overlap with those seen in the different clinical presentations of frontotemporal lobar degeneration (FTLD) or non-degenerative disorders such as psychiatric conditions. This leads to frequent misdiagnosis, as revealed in clinicopathological studies (Galton et al., 2000; Alladi et al., 2007; Ling et al., 2010; Balasa et al., 2011). Clinicopathological correlation studies have shown that the sensitivity and specificity of the clinical criteria for AD diagnosis are around 85% and 70% respectively (Beach et al., 2012). Recently, new diagnostic criteria for mild cognitive impairment (MCI) (Albert et al., 2011), AD (McKhan et al., 2011) and FTLD variants (Rascovsky et al., 2011; Gorno-Tempini et al., 2011) have been formulated; they incorporate biomarkers (neuroimaging, CSF or genetics) that improve the diagnostic accuracy.

The plausible possibility that disease-modifying drugs need to be administered early in the course of neurodegenerative diseases such as AD in order to be effective emphasizes the need for specific biomarkers to increase certainty in the diagnosis of the underlying pathological process. Different studies have demonstrated a good correlation between, on the one hand, cerebrospinal fluid (CSF) Aβ1-42 (Aß42) and tau protein levels; and on the other, pathological findings in AD patients (Josephs et al., 2008; Tapiola et al., 2009; Shaw et al., 2009). Many studies have likewise demonstrated that most AD patients present a typical profile of low Aß42 and high total tau (t-tau) and phosphorylated tau (p-tau); and furthermore this profile shows high sensitivity and specificity in differentiating AD from other neurodegenerative disorders or controls (Mattsson et al., 2009). In addition, most MCI subjects who subsequently progress to AD dementia already presented the typical AD CSF profile at onset of MCI (Buckhave et al., 2012). The robust evidence of the validity of CSF results made it possible to include the CSF biomarker profile in the recently revised criteria for MCI, AD and FTD for increasing the level of certainty of underlying AD pathology in MCI due to AD or AD dementia (Albert et al., 2011; McKhan et al., 2011) and ruling out an FTD diagnosis in case of positive results (Rascovsky et al., 2011).

Although frequent misdiagnosis and/or late diagnosis in EOAD would support the use of biomarkers in clinical practice, most of the CSF validation studies have been performed on subjects with late-onset cognitive decline, with few cohort studies in EOAD (Schoonenboom et al., 2004)

Our aims were to evaluate the usefulness of CSF biomarkers for an early AD diagnosis in a prospective clinical cohort of early-onset cognitive impairment by evaluating the diagnostic performance of CSF biomarkers for the differential diagnosis of EOAD and FTLD, in both amnestic and non-amnestic presentations; to assess the contribution of these biomarkers to the change in the level of certainty of the diagnosis and the prediction of conversion to AD dementia in subjects with MCI.

Material and methods

Subjects

Participants were recruited prospectively from the Alzheimer's Disease and Other Cognitive Disorders Unit outpatient clinic at the Hospital Clinic, Barcelona, Spain. All the participants gave written informed consent and the study was approved by the hospital's Ethics Committee. All *de novo* subjects who were referred with cognitive impairment between January 2009 and March 2013, with an age at onset below 65 years, were offered the option of participating in the project. To the usual clinical workup that includes clinical and complete neuropsychological evaluations, and a structural MRI, it added a spinal tap to determine AD biomarkers and genetic markers (APOE genotyping, serum progranulin levels, and genetic screening of pathogenic mutations in case of familial history of disease or particular phenotypes). Patients with normal structural neuroimaging and a doubtful diagnosis also underwent functional neuroimaging (FDG-PET or HMPAO-SPECT). Subjects with motor neuron disease at inclusion or with a condition that contraindicated the lumbar puncture were not included. Controls were also recruited as research volunteers and defined as individuals with no cognitive complaints and no evidence of cognitive impairment in any of the neuropsychological tests administered. 157 subjects were included in the study, with 37 controls (mean age 53y, mean MMSE 28.6) and 120 patients with early-onset cognitive impairment (mean age at inclusion 57.7y, mean age at onset 54.6y, MMSE 23.5).

Clinical classification

An initial clinical diagnosis was established prospectively based on the initial clinical and neuropsychological assessments as well as neuroimaging, using the diagnostic criteria current at the time of approval of the project (January 2009). The diagnostic categories were: MCI (Petersen et al., 2004) using the total delayed recall score of the Free and Cued Selective Reminding Test to diagnose episodic memory impairment (Grober et al., 1988; Pena-Casanova et al., 2009), AD (McKhann et al., 1984), primary progressive aphasia (Mesulam et al., 1982), posterior cortical atrophy (Tang-Way et al., 2004) and clinical variants of FTLD (Neary et al., 1998). Some patients also met the criteria for a psychiatric disorder, mostly dysthymic disorder (DSMIV-TR) but were included, as previous studies have reported that many early-onset patients receive a first diagnosis of a psychiatric disorder. In order to obtain a homogenous sample, these criteria were maintained and applied to the whole sample despite new diagnostic criteria for the most common neurodegenerative disorders being published during the inclusion period.

Additionally, during the final months of the study, all the patients were re-evaluated by three behavioral neurologists and an up-to-date diagnosis was established based on the new diagnostic criteria, follow-up and ancillary tests (except for the CSF results). This final diagnosis was established following the revised diagnostic criteria for MCI due to AD (Albert et al. 2011), AD dementia (McKhan et al., 2011), FTD (Raskovsky et al., 2011) and primary progressive aphasia (Gorno-Tempini et al., 2011).

The available biomarkers were classified as biomarkers of amyloidosis (Aβ42 CSF levels) and biomarkers of neuronal injury (structural MRI, FDG-PET and CSF t-tau and p-tau levels) as previously described (Albert et al., 2011; McKhan et al., 2011).

CSF biomarkers determination and results interpretation

All the subjects underwent a spinal tap, during the morning. The samples were centrifuged and stored in polypropylene tubes at −80ºC within 2 h. Levels of Aβ42, t-tau, and p-tau were measured using commercial sandwich ELISA kits (Innogenetics, Gent, Belgium). Experienced laboratory personnel who were blinded to the clinical diagnosis performed the tests. The laboratory in which the samples were processed participates in an Alzheimer's Association external quality control program for standardization of analytical procedures for

CSF biomarkers, and all the values were within mean program values ± 2 SD (Mattsson et al., 2011).

First, the diagnostic value for discriminating between AD subjects and controls (optimal threshold values, sensitivity and specificity) were evaluated for the different individual biomarkers (Aβ42, t-tau and p-tau) as well as composite biomarkers such as the Aβ42/p-tau ratio. In this step, we selected only samples from subjects with an AD diagnosis both at inclusion and at the final evaluation. In a second step, the biomarker thresholds established previously were applied to the other subjects (MCI and FTLD) and we classified each participant as CSF positive (if the CSF value supported AD pathology) or negative.

Mean CSF values were evaluated in the different diagnostic categories (amnestic and non-amnestic presentation of AD, FTLD and MCI). We then assessed how the addition of CSF to the clinical and neuroimaging data changed the likelihood of AD pathophysiological in MCI or AD patients, and how it affected the probability of an FTLD diagnosis. As proposed by the new criteria, the diagnostic probability of an underlying AD pathophysiology was considered: high, if both amyloidosis and neuronal damage biomarkers were positive; intermediate, if one biomarker was positive and the other untested or had uninformative results for the diagnosis; low or probably not due to AD pathology, if both amyloidosis and neuronal damage biomarkers were negative; or uninformative, in cases of conflicting results between amyloidosis and neuronal damage biomarkers. These probabilities were assessed in the AD and MCI groups both without and with the CSF results.

Finally, we evaluated the capacity of the CSF to predict clinical progression to AD dementia during follow-up in the MCI group, and compared clinical and ancillary data between those MCI who developed AD dementia and those who remained stable.

Biochemical and genetic biomarkers

Blood samples for DNA extraction and serum collection were taken from all the subjects. The *APOE* genotype was determined using PCR amplification and the HhaI restriction enzyme. A commercial ELISA kit was used to assess the serum progranulin levels (Adipogen, Seoul, South Korea) according to the manufacturer's specifications. All patients with serum progranulin levels < 120 ng/mL were screened by direct sequencing for the presence of *GRN* mutations as described previously (Antonell et al., 2012). Mutation

screening for *PSEN1*, *PSEN2*, *APP* and *MAPT* was performed on all subjects with an autosomal dominant pattern of inheritance. Subjects with a family history of FTLD or motor neuron disease were also screened for *C9orf72* expansion.

Statistical analysis

The data were analyzed with the SPSS package for Windows (v.19.0, IBM Corp., NY, USA). T-test analysis was used when comparing quantitative variables and χ^2 when comparing categorical data. When a data distribution was not normal, non-parametrical tests were used. The diagnostic power of the different biomarkers was evaluated by receiver operating characteristic (ROC) analysis.

Results

Initial clinical classification

The initial clinical diagnosis, demographics and *APOE* genotype for the different categories are displayed in Table 1. There were 51 MCI (25 amnestic, 20 amnestic multidomain and 6 nonamnestic), 42 AD, 10 bvFTD, 3 posterior cortical atrophy and 14 primary progressive aphasia cases.

Neuroimaging

For the purposes of this study, we dichotomized the clinical evaluation of the MRI images into positive if the pattern of atrophy supported the clinical diagnosis and negative if it was considered normal or uninformative for the clinical diagnosis. A positive MRI for AD was considered when the patient presented disproportionate atrophy in medial, basal, and lateral temporal lobe, and medial parietal cortex; for FTD when presented a disproportionate frontal and/or anterior temporal atrophy and for language variants of frontotemporal lobar degeneration when predominant left posterior fronto-insular atrophy or predominant anterior temporal lobe atrophy was observed. Forty-five (37.5%) patients underwent FDG-PET /HMPAO-SPECT and a similar approach was used for classifying the results as positive and negative.

Genetic testing results

Eight subjects carried a *PSEN1* mutation. All of them had a family history of AD with an autosomal dominant pattern of inheritance and a typical amnestic presentation of symptoms. Two *MAPT* mutations were identified in subjects with bvFTLD and semantic dementia diagnoses. Three subjects (1 FTD and 2 PPA) carried a pathological hexanucleotide expansion in *C9orf72*. We also identified three probable pathogenic *GRN* mutations in subjects with very low PGRN levels (1 patient clinically classified as AD and 2 PPA). It is noteworthy that these three subjects presented the lowest progranulin levels of the sample. There were no differences between progranulin levels between AD, controls, MCI and FTLD (once the *GRN* mutation carriers had been excluded).

Final clinical diagnosis

The mean follow-up time of the patients included in this study was 25 months, (SD 15.8 months, median 25.8 months, range 1.5-59.5 months). Twenty-five patients received a final diagnosis of MCI, 4 subjects who were initially diagnosed as MCI had normal cognition and 66 fulfilled core clinical criteria for AD dementia according to NIA-AA criteria of 2011 (including 11 non-amnestic presentations (17%): 5 language variants, 4 posterior variants and 2 frontal variants). (See Table 2 for detailed demographics).

A total of 25 patients fulfilled the criteria for one of the FTLD variants (11 FTD, 5 semantic variant and 9 non-fluent variant of PPA).

When comparing initial and final diagnosis, most AD patients (37 patients, 88%) maintained the core clinical criteria for AD diagnosis according to the revised criteria. Of the other 5: one patient who met the AD criteria at inclusion (McKhann, 1984) carried a *GRN* mutation and was reclassified as FTLD; the cognition of two subjects improved and while at final diagnosis they met the clinical criteria for MCI, they did not meet the AD dementia criteria; and two subjects had a clinical and neuroimaging evolution compatible with FTD.

Threshold values of CSF biomarkers

The descriptive values of the different CSF biomarkers in the different diagnostic groups are detailed in Table 2. As expected, the AD group had lower Aβ42 levels and Aβ42/p-tau ratios, and higher t-tau and p-tau values than any other group ($p < 0.001$). Threshold values with the corresponding sensitivities and specificities, as well as AUC based values on ROC curves, that best discriminated between AD and controls in our cohort were subsequently calculated. As previously mentioned, to determine the thresholds, only patients who fulfilled the diagnostic criteria for probable AD dementia both at inclusion (McKhan el al., 1984) as well as the core clinical criteria at the end of the study (Mc Khan et al., 2011) were included. The biomarker that best predicted in the cohort was the Aβ42/p-tau ratio. A threshold of 6.7 gave 92% sensitivity and 94.6% specificity, with an AUC of 0.944; thereby outperforming every individual biomarker. This value was then used in order to classify the subjects as CSF positive (Ratio Aβ42/p-tau < 6.7) or negative (Ratio Aβ42/p-tau ≥ 6.7). The diagnostic values of individual biomarkers as well as the Aβ42/p-tau ratio are displayed in Table 3.

CSF results within groups

All the subjects with non-amnestic presentations of AD and all but two subjects with amnestic presentation of AD had a positive CSF pattern. There were no differences in baseline demographics of CSF biomarkers between typical and atypical AD presentations. All but one subject in the final FTLD group showed negative CSF. Two controls had positive CSF results.

MCI subject follow-up and CSF characteristics at baseline

On the initial evaluation, 51 subjects met Petersen's criteria for MCI. During follow-up (mean 30.3 months, median 32 months, range 2-59 months): 24 of them (47%) progressed clinically and met criteria for AD dementia; 23 (45%) remained stable; and 4 (8%) improved to normal cognition. There were two patients initially diagnosed as AD whose cognition improved and at final diagnosis met the criteria for MCI; both of them showed negative CSF at baseline. The clinical evolution of the MCI subjects and the relation with the CSF status at baseline are displayed in Figures 1 and 2.

All the MCI subjects who subsequently progressed to AD dementia had positive CSF results at baseline (Aβ42/p-tau ratio < 6.7). Specifically, 4 subjects had low Aβ42 with normal t-tau or p-tau levels; one subject had normal Aβ42 with altered t-tau and p-tau; one subject had uninformative (borderline) Aβ42 with pathological t-tau and p-tau; and the remaining 18 had both amyloidosis and abnormal neuronal damage biomarkers. 88% of the MCI patients that progressed to AD dementia had positive neuronal damage neuroimaging biomarkers at baseline and 50% of them carried at least one APOE ε4 allele. In contrast, all but one of the stable MCI subjects or those who subsequently improved had negative CSF at baseline (the only MCI patient with positive CSF and no progression over time, had a very short follow-up time of less than 3 months). Individual results showed normal Aβ42 and tau in 20 subjects, uninformative (borderline results) Aβ42 in three, uninformative tau in one, low Aβ42 in one and low Aβ42 and high tau in one patient. Only 11% of the stable MCI patients had positive neuronal damage imaging biomarkers at baseline (MRI and/or FDG-PET) and only 23% of them were APOE ε4 positive. There were no baseline differences between stable MCI and subjects with normalization of cognition during follow-up.

The follow-up period for stable MCI subjects was inferior to that of those who progressed to AD ($p= 0.02$) with no significant differences in age at onset, age at first evaluation or baseline MMSE scores (Figure 1).

Changes in the probability of underlying AD pathology after incorporating biomarkers results

In order to evaluate the impact of the biomarkers (CSF and neuroimaging) on the degree of confidence in the diagnosis, the changes in the probability of underlying AD pathology were evaluated before and after adding biomarker results to the core clinical criteria within the different diagnostic categories.

With neuroimaging data (structural MRI and FDG-PET/SPECT) added to the core clinical criteria, 10 out of 55 subjects with amnestic presentation of AD had uninformative neuroimaging results and 45 presented an intermediate likelihood of AD pathology (see Table 4). Of the nonamnestic presentations: 2 subjects had uninformative neuroimaging and 9 an intermediate likelihood of AD pathology. Twenty-three MCI subjects presented uninformative neuroimaging regarding AD etiology and two an intermediate likelihood.

Within the FTLD group, 21/25 (84%) had neuroimaging findings that supported probable FTLD diagnosis.

The addition of the CSF results to the core clinical criteria and neuroimaging data increased the likelihood of underlying AD pathology to the level of high probability in 49 (90%) of typical AD subjects and 9 (82%) patients with atypical AD (see Table 4 for details). In contrast, normal CSF results correlated with a low probability of underlying AD pathology (or MCI probably not due to AD) in 76% of the patients with MCI at the end of the follow-up (non-converters).

Within the FTLD group, one subject (diagnosed clinically as possible FTD) had a clearly positive CSF result; it should be noted that this subject lacked supportive MRI. Three other subjects had relatively low Aβ42 levels with normal t-tau and p-tau, and a normal Aβ42/p-tau ratio.

Discussion

We demonstrated in a prospective sample of early-onset cognitive impairment subjects that CSF biomarkers differentiate EOAD and FTLD with high sensitivity and specificity. The revised NIA-AA criteria for AD dementia can reliably diagnose probable AD in both amnestic and non-amnestic presentations; and the CSF biomarkers increased the certainty of the clinical diagnosis to a high likelihood in most cases in both presentations. Finally, the vast majority of early-onset MCI subjects who will progress to AD dementia within 3 years had pathological baseline CSF results.

Patients with EOAD, especially those with non-amnestic presentations, are often misdiagnosed if the diagnosis is solely based on clinical data (Alladi et al., 2007; Balasa et al., 2011), leading to suboptimal sensitivity and specificity of clinical diagnostic criteria when tested in pathological confirmed subjects (Knopman et al., 2001; Beach et al., 2012). One of the great steps forward of the new NIA-AA criteria for AD dementia was the recognition of non-amnestic presentations and their inclusion in the diagnostic criteria for probable AD. These criteria also acknowledge the role of different biomarkers in increasing the probability that the cognitive impairment is caused by AD pathology. The fact that, in our cohort, in subjects fulfilling clinical diagnostic criteria for probable AD, there were no significant difference in CSF biomarkers between amnestic and non-amnestic presentations

supports the use of these biomarkers for increasing the certainty of the diagnosis regardless of the clinical presentation.

The CSF biomarker that best discriminated in our cohort between AD and control subjects was a composite measure: the Aβ42/p-tau ratio. This is not surprising as previous work also supports combining the two main categories of AD biomarkers (amyloidosis and neurodegeneration) in a single value to increase both sensitivity and specificity, as it is less likely that non-AD subjects present with a similar profile (Fagan et al., 2007; Welge et al., 2009; Buckhave et al., 2012). Previous papers suggest that it is early-onset dementia subjects that could potentially benefit most from the use of CSF biomarkers, because of the lower frequency of pathological CSF in age- matched control subjects with respect to older control groups with an increased positive predictive value of an altered biomarker (Bouwman et al., 2009; Antonell et al., 2010). Only 8% of our controls had a diminished Aβ42 CSF level and even fewer (5%) had an altered composite Aβ42/p-tau ratio biomarker.

Pathological studies also indicate that a higher percentage of older people with dementia have mixed pathological findings as the main cause of their cognitive impairment than is the case in younger subjects implying a higher predictive value of a positive AD biomarker in younger subjects (Schneider et al., 2007; Savva et al., 2009).

With respect to the usefulness of CSF biomarkers for EOAD and FTLD differential diagnosis, although most patients within each group had a suggestive clinical picture with compatible neuroimaging or genetic data, the presence of clearly pathological CSF results combined with the lack of specific radiological findings supported the presence of AD pathology in 6 subjects. In contrast, in the present cohort as well as in previous publications, some genetic FTLD cases, such as carriers of *GRN* mutations (van Swieten et al., 2008; Perry et al., 2013) or hexanucleotide expansions in *C9orf72* (Wojtas et al., 2012) can be clinically misdiagnosed as AD. In both these groups of patients, the study of CSF biomarkers could offer a correct final diagnosis. Previous studies emphasized the added value of CSF AD biomarkers in well characterized clinical cohorts in increasing the diagnostic certainty or producing a change of clinical diagnosis in a significant percentage of patients (Kester et al., 2010). Larger cohorts used to evaluate CSF biomarkers showed good sensitivity but relatively low specificity, especially in differential diagnosis with vascular cognitive impairment or Lewy body disease (Schoonenboom et al., 2012). Those data are of particular importance as the conditions are highly infrequent in early-onset dementia, supporting once again a higher predictive value of a positive result.

Previous longitudinal studies reported that baseline alteration of CSF biomarkers can predict with high probability which MCI patients will subsequently decline and progress clinically to AD dementia (Buckhave et al., 2012). We have confirmed those findings in a sample composed exclusively by subjects with early-onset of the symptoms. The fact that 96% of MCI with pathological CSF at baseline are currently diagnosed as AD dementia after a median follow-up of approximately three years underlines the great utility of the procedure.

One limitation of our results is that the stable MCI subjects underwent significantly shorter follow-up than those who progressed to AD dementia; although it was longer than 2 years. This raises the possibility that some of them could progress to AD dementia or other neurodegenerative diseases with time. Although that is a possibility that we cannot rule out completely, it seems highly unlikely to us. Those subjects not only had a negative baseline CSF, most of them also lacked positivity in neuroimaging neuronal damage biomarkers, none had relevant vascular damage and they presented significantly less APOE ε4 than those patients who got worse during follow-up. Many of them also met the criteria for a psychiatric disorder (mainly affective disorders) at first evaluation or during follow-up and it was presumably the cause of their cognitive deficits. These data together lead us to believe that the differences in clinical progression between positive and negative CSF MCI groups are real and not due to insufficient follow-up. Another limitation when interpreting the data from the present study is the lack of pathological confirmation of the diagnosis. However, the presence of a significant number of mutation carriers in the different groups and the use of complementary examinations used for clinical classifications (MRI or FDG-PET) together with the clinical follow-up, increases our confidence that the classification approached the "underlying biology". We acknowledge that the use of an amyloid-PET tracer in the cohort could have resulted in similar results in strengthening the probability of underlying AD pathophysiological process.

In conclusion, CSF biomarkers seem to be extremely useful in the differential diagnosis of early-onset dementia by discriminating AD from FTLD subjects with high sensitivity and specificity. Regardless of the specific AD clinical presentation, CSF biomarkers helped to increase the probability of an accurate AD diagnosis. Baseline CSF also predicts subsequent impairment and progression to AD dementia with high accuracy in subjects with early-onset MCI.

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	MCI	AD	FTD	PCA	PPA	CTR
N	51	42	10	3	14	37
Age at first evaluation (SD)	57.9(6)	57.7(6)	54.8(6)	58(1.7)	58.6(6)	53 (9)
Men/Women	28/23	18/24	7/3	2/1	7/7	7/30
AAO (SD)	55(6)	54.6(6)	51(5)	54.3(1.5)	56(6)	-
MMSE (SD)	25.6 (2.6)	20(4.7)	22(5.7)	25.5(2)	27.6 (2)	28.6 (1.6)
APOε4 +	37.5%	42.5%	30%	33%	30%	24%

Table 1 Demographics of initial clinical diagnosis categories. All values are displayed as mean (SD). Abbreviations: MCI, mild cognitive impairment; AD, Alzheimer's disease; FTD, behavioral variant of frontotemporal dementia; PCA, posterior cortical atrophy; PPA, primary progressive aphasia; CTR, controls; AAO, Age at clinical onset; MMSE, baseline MiniMental State Examination score.

	Stable MCI	AD	FTLD	MCI that normalized cognition	CTR
N	25	66	25	4	37
Age at first evaluation (SD)	57.2 (4.8)	58.4 (6.2)	56.8 (6)	55 (7.2)	53(9)
Men/Women	15/10	28/38	15/10	4/0	7/30
AAO (SD)	54.4 (5.4)	55.2 (6.2)	54 (6)	52.8 (8)	-
Initial MMSE (SD)	26 (2.3)	22.4 (4.6)	23.1 (5.8)	28.5 (1.5)	28.6 (1.6)
APOε4 +	26%	45%	20%	24%	24%
Follow-up period in months (SD)	23.4 (15)	27.6 (15)	20.6 (15)	29 (4)	24.2 (18)
Aβ42	677 (190)	360 (136)	695 (163)	643 (84)	710 (183)
Mean (SD), Range	344-1158	148-816	436-991	580-766	262-1087
T-tau	258 (223)	755 (382)	335 (236)	144 (37)	260 (160)
Mean (SD), Range	91-1250	150-1600	70-1200	98-188	103-1060
P-tau	51(24)	113 (67)	49 (19)	38 (7)	56 (24)
Mean (SD), Range	21-144	33-540	14-96	30-48	26-154
Aβ42/p-tau ratio	14.8 (4.9)	4 (3.1)	16(6.8)	17 (1.9)	13.7 (3.7)
Mean (SD), Range	2.4-25	1-24	6.2-43	16-20	3.9–22.7

Table 2 Demographics, APOE genotype distribution and CSF biomarker results in the final current diagnostic categories. All values are displayed as mean (SD). Abbreviations: MCI, mild cognitive impairment; AD, Alzheimer's disease; FTLD, Frontotemporal dementia

variants (Behavioural, non-fluent and semantic variants of primary progressive aphasia); AAO, Age at clinical onset; MMSE, baseline MiniMental Score; CTR, controls; t-tau, total tau; p-tau, phosphorylated tau. The CSF biomarkers values are expressed in pg/ml.

	Threshold	Sensitivity	Specificity	AUC	AUC IC (95%)
Aβ42	486 pg/ml	86.5%	89.2%	0.916	0.847 - 0.985
t-tau	365 pg/ml	83%	94.6%	0.910	0.834 – 0.987
p-tau	59 pg/ml	86.5%	75.7%	0.859	0.771 – 0.948
Aβ42/p-tau	6.7	92%	94.6%	0.944	0.881 - 1

Table 3 Diagnostic performance of individual and composite CSF biomarkers for the differential diagnosis of AD and controls. Abbreviations: t-tau, total tau; p-tau, phosphorylated tau; AUC, area under curve; IC, interval of confidence

Revised diagnosis (core clinical criteria)	Biomarker probability of AD etiology	Core clinical criteria + neuroimaging	Core clinical criteria + neuroimaging + CSF
Typical AD (N=55)	Uninformative	**10**	**3**
	Intermediate	**45**	**1**
	High	**0**	**49**
	Probably not due to AD	**0**	**2**
Atypical AD (N=11)	Uninformative	**2**	**2**
	Intermediate	**9**	**0**
	High	**0**	**9**
	Probably not due to AD	**0**	**0**
MCI (N=25)	Uninformative	**23**	**5**
	Intermediate	**2**	**0**
	High	**0**	**1**
	Probably not due to AD	**0**	**19**

Table 4 Contribution of CSF biomarkers to the changes in the likelihood of an underlying AD pathology in AD and MCI groups diagnosed with revised clinical criteria (McKhan et al. 2011, Albert et al. 2011).

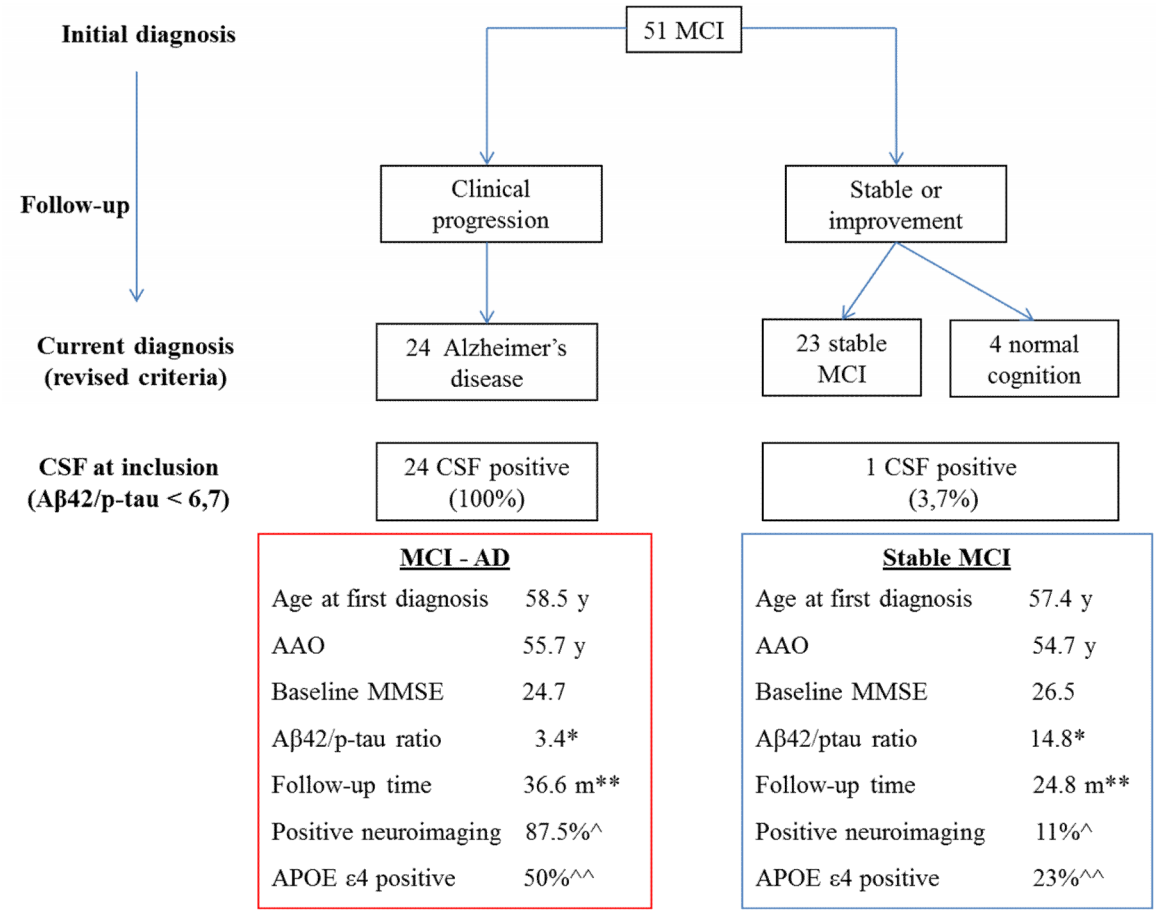

Figure 1 Clinical evolution of mild cognitive impairment subjects and their relations with CSF status. Abbreviations: CSF positive, Aβ42/p-tau ratio < 6.7; CSF negative, Aβ42/p-tau ratio ≥ 6.7; AAO, age at onset; * p = 0.02; ** p = 0.001; ^ p = 0.04; ^^ p = 0.001

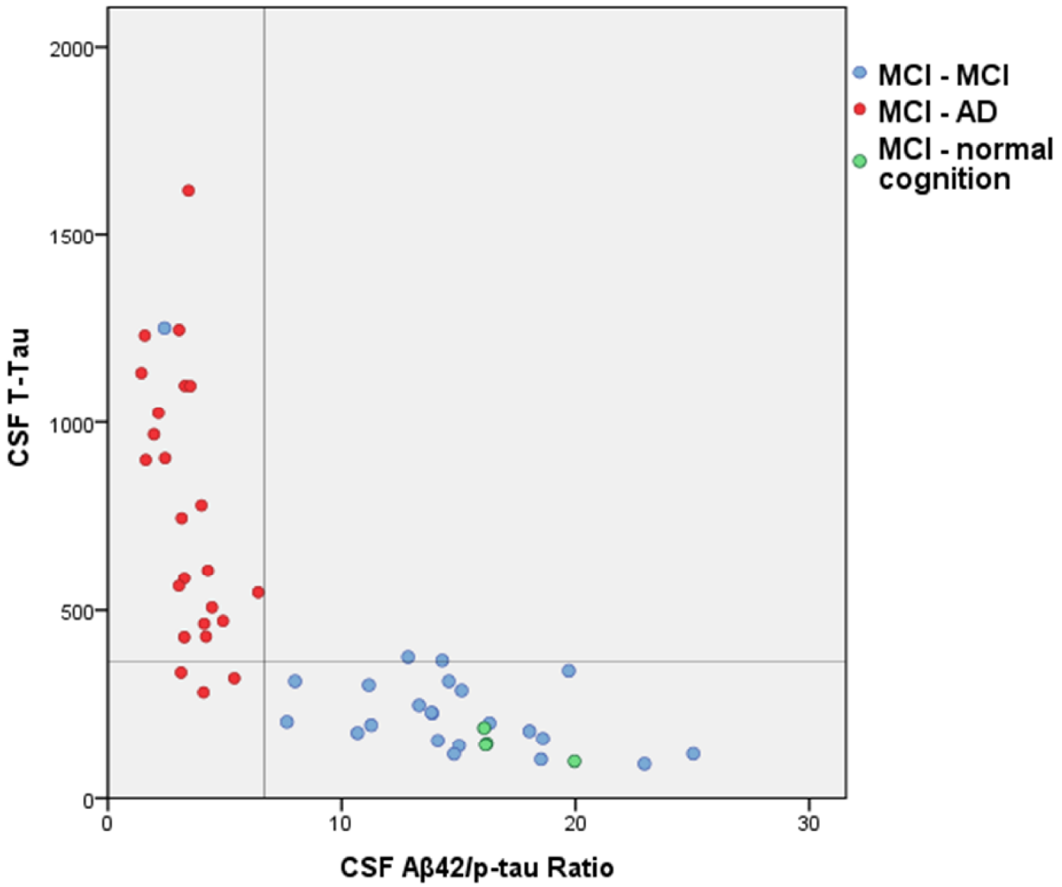

Figure **2** Baseline CSF at initial MCI diagnosis and subsequent clinical evolution. X Axis reference line represents the CSF Aβ42/p-tau Ratio threshold of 6.7 and the Y axis reference line the threshold for t-tau (365 pg/ml).

Trabajo número 5

A novel *PSEN1* gene mutation (L235R) associated with familial early-onset Alzheimer's disease

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A novel *PSEN1* gene mutation (L235R) associated with familial early-onset Alzheimer's disease

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ABSTRACT

Mutations in the presenilin 1 (*PSEN1*) gene are the most frequent cause of familial Alzheimer's disease (AD), with at least 182 different mutations published to date. We report a 48-year-old woman (age at onset 47 years) who presented a progressive alteration of episodic memory, spatial disorientation, apathy, language disturbances and neglect of personal care. Her MMSE score was 20/30. The patient presented an unusually rapid deterioration and at 6 months follow-up her cognitive and functional status had worsened considerably (MMSE score of 11). Cranial MRI showed a bilateral atrophy with temporal and parietal predominance and the quantification of AD CSF biomarkers showed the typical AD signature. Family history evidenced an autosomal dominant pattern of inheritance. Mutational screening was performed by direct sequencing of exons 3–12 of *PSEN1*. The patient presented the 3/3 *APOE* genotype. Genetic analysis revealed a nucleotide substitution in exon 7 of *PSEN1* gene, producing a missense mutation in codon 235 from leucine amino acid to arginine (L235R). This amino acid is conserved between presenilin-1 and presenilin-2 proteins. The L235R mutation had not been previously reported, although other mutations in the same residue have also been associated with familial early-onset AD, providing support for the importance of this residue for the presenilin-1 function.

Alzheimer's disease (AD) is the most frequent cause of dementia worldwide. In most cases it is a multifactorial disease. However, slightly less than 1% of cases are monogenic with an autosomal dominant pattern of inheritance. Mutations causing genetic AD have been described in three genes: *PSEN1*, *APP* and *PSEN2* [6]. To date, 182 mutations have been reported for *PSEN1* in the Alzheimer Disease & Frontotemporal Dementia Mutation Database (AD&FTDMDB) (http://www.molgen.ua.ac.be/admutacions/). The penetrance of the mutations is almost complete by age 65 and the clinical presentation resembles sporadic AD, but some present particular phenotypes [8]. In this study, we describe a novel *PSEN1* mutation associated with familial early-onset AD with a rapid clinical progression.

A 48-year-old right-handed woman without formal education consulted for cognitive impairment. At 47 years she had begun to have episodic memory problems. Her family reported forgetfulness (losing objects and forgetting meetings, conversations, and receipts), diminished language fluency and word-finding difficulties. There were also some behavioural disturbances such as apathy and self-neglect. At the first examination she presented a MiniMental State Examination (MMSE) score of 20/30. She was referred to the Alzheimer's Disease and Other Cognitive Disorders Unit and was seen six months after the first neurological evaluation. At this visit, she could name only four animals in one minute, was unable to draw a clock, and her MMSE score had dropped to 11. The cognitive evaluation showed severe alterations of fixation, working memory, verbal and visual episodic and semantic memory. She presented notable executive deficits, generalised apraxia and anomia and alteration of visuospatial and visuoperceptual complex functions. No gait disturbances, pyramidal or extrapyramidal signs or myoclonus were observed. She underwent cranial Magnetic Resonance Imaging (MRI) revealing bilateral atrophy with temporal and parietal predominance (Fig. 1), and a lumbar puncture for AD CSF biomarkers quantification. Aβ1-42-amyloid peptide ($A\beta_{42}$), total tau protein (t-tau) and phosphorylated tau at threonine 181 (p-tau_{181}) were measured by ELISA as previously described [1]. In the local laboratory, the pathologic cut-off values are $A\beta_{42}$ < 500 pg/mL, t-tau > 300 pg/mL and p-tau_{181} > 75 pg/mL ([11] and own unpublished data). The patient presented the typical AD biomarker signature: low $A\beta_{42}$

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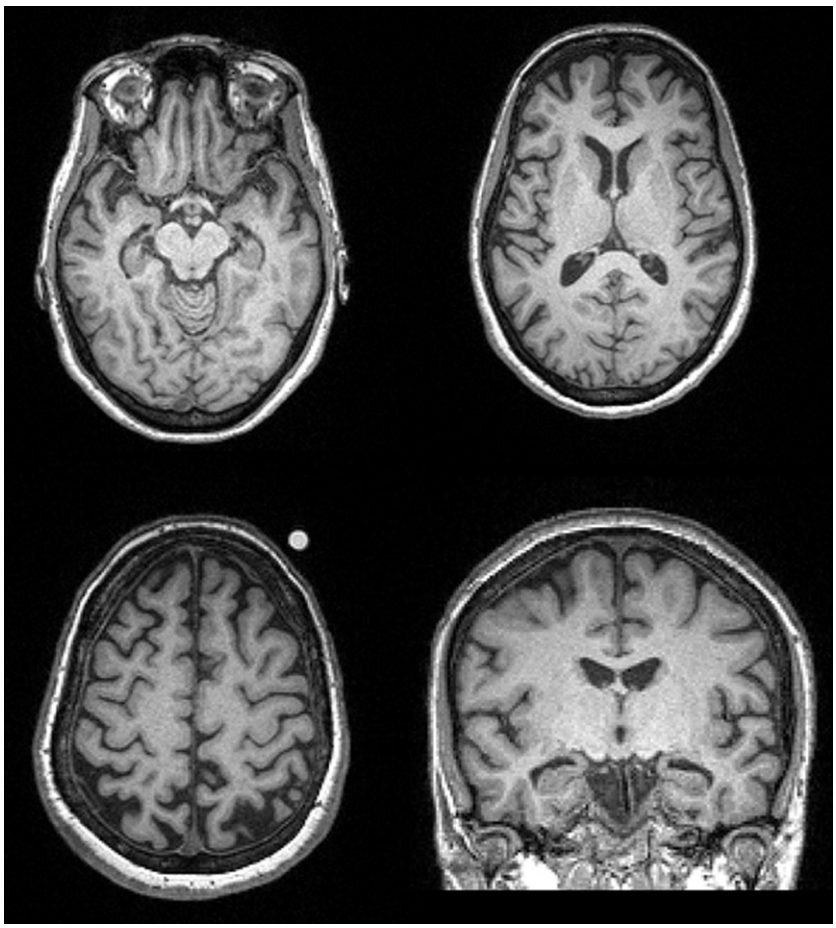

Fig. 1. 3 T MRI (T1 3D) showing moderate bilateral temporal and parietal atrophy.

(279.1 pg/mL), elevated t-tau (1532.3 pg/mL) and elevated p-tau$_{181}$ (172.2 pg/mL).

Her family history revealed seven additional cases of early-onset dementia in two generations with a pattern of autosomal dominant inheritance (Fig. 2). Mean age at onset was 45 years (range 41–60 years). Although extensive clinical information was not available for most of the relatives, a cousin of the proband presented a disease duration of three years from disease onset until death (44–47 years), while another cousin had a MMSE score of 18 only one year after disease onset (onset at 41 years), presenting already moderate dementia and myoclonus. A clinical diagnosis of familial early-onset AD was established.

Genetic analysis of the proband was undertaken after genetic counselling and written informed consent [9]. Coding exons 3–12 of *PSEN1* were analysed by direct sequencing as previously described [3]. *PSEN2* and *APP* genes were not screened for the presence of further mutations. *APOE* genotyping was performed by PCR amplification and *HhaI* restriction enzyme digestion.

Sequence analysis of exon 7 of *PSEN1* identified a heterozygous T to G transversion in codon 235 (c.704T>G) (Fig. 3). This mutation predicts an amino acid change from leucine (L) (CTG) to arginine (R) (CGG), leading to the L235R missense mutation. This mutation was not recorded in the AD&FTDMDB or in The Human Genetics Mutation Database at the Institute of Medical Genetics

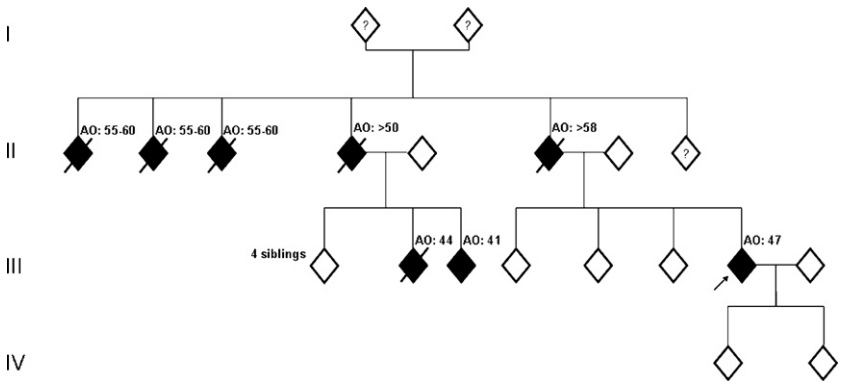

Fig. 2. Pedigree of the family described bearing the L235R mutation in *PSEN1*. Sex has been omitted to protect confidentiality. AO, age at onset. Arrow indicates the proband.

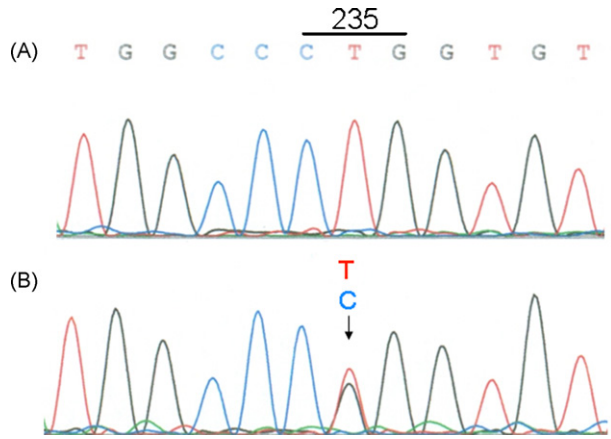

Fig. 3. Electropherogram showing a fragment of the DNA sequence of exon 7 of *PSEN1* showing a missense mutation T→C at the second position of codon 235 (arrow), which predicts a leucine-to-arginine substitution from a normal sequence in a control (A) and in the proband (B).

in Cardiff (HGMD) (http://www.hgmd.cf.ac.uk/ac/index.php). To predict the impact of the novel variant on protein function, the recently proposed algorithm [6] and PolyPhen program (http://genetics.bwh.harvard.edu/pph) were used. The PolyPhen program score (1.976) predicted the variant as possibly damaging to protein structure and function. The proband presented the *APOE* 3/3 genotype.

We describe a new mutation in the *PSEN1* gene which involves a change of amino acid at codon 235 from leucine to arginine: L235R. Codon 235 is located in transmembrane domain five (TM-V), where seven different mutations have already been described, representing 9.39% of the total of mutations in *PSEN1*. An L to R change would be expected to disrupt the transmembrane domain architecture in the protein. This sequence variant in *PSEN1* was absent in 121 controls and 231 patients with AD from the Iberian peninsula [6], occurred in a residue where two other mutations have previously been reported [2,4,7,10] and the Leu residue at 235 in PSEN1 is conserved in PSEN2 protein.

Mild behavioural symptoms are frequent in presenile AD and present in many *PSEN1* mutations at some stage of the disease. In contrast, features such as highly aggressive course appear associated only with specific mutations. In general, the codon affected has a major influence on the clinical phenotype, while the amino acid-type substitution is less important [5]. Our patient presented rapid progression (a fall of 9 points in the MMSE score in 6 months) associated with low levels of $A\beta_{42}$ and very high levels of t-tau and p-tau_{181}. Low levels of $A\beta_{42}$ and very high levels of CSF t-tau and p-tau_{181} have been associated with a faster progression of cognitive deficits and higher mortality [12].

The L235V mutation has been described in one family with a mean age at onset of 47 years (44–50) [7] and the L235P mutation in three different families with the phenotype of AD and myoclonus with a mean age of onset of 32.3 years [2,4,10]. The fact that mutations in this codon are associated either with a very early age of onset (in the L235P) or a fast clinical progression (our L235R) adds further support to the importance of this residue for the function of PSEN1.

Due to the lack of samples it was not possible to confirm the co-segregation of the mutation in affected relatives. However, following the algorithm key proposed by Guerreiro et al. [6] the mutation L235R can be classified as "Probable pathogenicity".

In conclusion, we describe a novel missense mutation in the *PSEN1* gene (L235R) of probable pathogenicity associated with autosomal dominant early-onset AD and rapid clinical progression.

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VII.

DISCUSIÓN

DISCUSIÓN

Los diferentes estudios que conforman la presente memoria de tesis doctoral han pretendido, a través de diversos diseños y metodologías, profundizar en el conocimiento de las características clínicas y biológicas de la enfermedad de Alzheimer de inicio presenil (EAP) esporádica, con la finalidad última de mejorar su diagnóstico precoz y desentrañar algunos de los procesos fisiopatológicos implicados en la enfermedad. Los resultados de los diferentes trabajos presentados aportan nuevos datos en la EAP, un campo restringido debido a su relativa baja prevalencia.

En primer lugar se han analizado las presentaciones clínicas de la EAP en nuestro entorno en una cohorte con confirmación neuropatológica, estableciendo los correlatos entre la presentación clínica, el diagnóstico clínico, los hallazgos neuropatológicos y el genotipo de *APOE* (trabajo 1). El relevante porcentaje de pacientes con una EAP con confirmación neuropatológica que no recibieron un diagnóstico de EA en vida, especialmente en el subgrupo con presentaciones atípicas, objetivado en este estudio de correlación clínico-neuropatológico, subraya la limitación de los criterios diagnósticos clínicos clásicos (McKahn et al., 1984) y la necesidad de utilizar biomarcadores para intentar mejorar el diagnóstico clínico. Así, en el trabajo 4 se ha evaluado en nuestro medio, el uso de biomarcadores en una cohorte prospectiva de pacientes con deterioro cognitivo de inicio presenil de diferentes etiologías, demostrándose su aplicabilidad y utilidad para mejorar el diagnóstico clínico de EAP, incluso en fases precoces de la enfermedad.

En los trabajos 2 y 3, se ha caracterizado la EAP esporádica desde el punto de vista de neuroimagen estructural por RM y de niveles de marcadores bioquímicos en LCR en una cohorte clínica, y de expresión génica a nivel cerebral en una cohorte neuropatológica, confrontándola con controles cognitivamente sanos y con pacientes con EAP genéticamente determinada por la presencia de mutaciones en el gen *PSEN1*. Los hallazgos de estos estudios apoyan el hecho de que, si bien los pacientes con mutaciones en *PSEN1* podrían tener una mayor carga de β-amiloide cerebral, ello no se ve reflejado en un mayor grado de neurodegeneración, al menos en la fase clínica de demencia sugiriendo que la neurodegeneración en esta etapa de la enfermedad en la EAP sería relativamente independiente del depósito de amiloide. Por otra parte, si bien se postula que las causas iniciales en la EAP esporádica serían diferentes a la EAP de causa genética por mutaciones en *PSEN1*, en ambas se objetiva una alteración en múltiples vías relevantes para el funcionamiento e integridad neuronal respecto a controles, sin que se puedan demostrar

alteraciones diferenciales en ambos subtipos de EAP, al menos en fases finales de la enfermedad.

Finalmente, en el trabajo 5 se describe una nueva mutación de patogenicidad probable en *PSEN1* detectada en el curso de los estudios realizados en población clínica con EAP.

El estudio de cohortes patológicas ha sido fundamental en la mejora del conocimiento en el campo de las demencias neurodegenerativas al permitir estudiar a sujetos con un diagnóstico definitivo. El trabajo de correlación clínico-patológica presentado (trabajo 1) aporta nuevos datos y confirma en pacientes de nuestro entorno los resultados obtenidos en otras cohortes patológicas. Aproximadamente un tercio de los sujetos con EAP esporádica tiene presentaciones clínicas atípicas, definidas como la ausencia de afectación relevante de la memoria episódica al inicio de la sintomatología. Las tres grandes categorías reconocidas de presentaciones atípicas de la EA son la presentación frontal, la presentación en forma de trastornos del lenguaje y las presentaciones posteriores (atrofia cortical posterior y síndrome corticobasal) (Dubois et al. 2010, McKhan et al. 2011). Los sujetos con presentaciones clínicas atípicas tienen zonas específicas de atrofia e hipometabolismo cortical que se relacionan con los síntomas, si bien comparten grandes áreas de atrofia cerebral con las presentaciones típicas (Migliaccio et al., 2009). La mayoría de la evidencia disponible actualmente apunta a que estas diferencias regionales de atrofia e hipometabolismo en presentaciones no-hipocámpicas (lenguaje o variantes posteriores) no se explican por una diferencia regional en la carga de amiloide fibrilar detectada *in vivo* (Rabinovici et al., 2008; Rosenbloom et al., 2011; de Souza et al., 2011). Esto implicaría que es posible que la causa inicial de la variación fenotípica pudiera ser diferente al depósito de amiloide y que los biomarcadores de depósito de amiloide podrían ser utilizados en igualdad de condiciones y rendimiento, tanto en las presentaciones típicas como atípicas, para apoyar el diagnóstico clínico.

La causa de la asociación entre EAP y una mayor frecuencia de presentaciones atípicas respecto a la EAT es desconocida, si bien podría estar relacionada con una mayor vulnerabilidad selectiva (de base genética y probablemente modulada por factores ambientales) de redes neuronales específicas a la agresión biológica de la enfermedad y depósito de proteínas anómalas que conducirían a fallos funcionales iniciales seguidos de neurodegeneración en regiones implicadas en tareas específicas como memoria, lenguaje o funciones visuoespaciales (Seeley et al., 2009).

A nivel neuropatológico en la cohorte analizada no encontramos diferencias en la distribución topográfica de patología neurofibrilar y amiloide entre las diferentes presentaciones clínicas si bien este análisis no era uno de los objetivos principales del trabajo y sólo se realizó una valoración semicuantitativa de la distribución de las lesiones. Existen, sin embargo, estudios previos de correlación clínico-patológica que han podido correlacionar la topografía de la carga neurofibrilar con los hallazgos clínicos. Así, se ha descrito una mayor carga neurofibrilar en áreas visuales primarias y áreas de asociación visual y menor en hipocampos en sujetos con atrofia cortical posterior respecto a casos típicos de EA (Tang-Wai et al., 2004); en sujetos con variantes de lenguaje hay una mayor cantidad de patología neurofibrilar en zonas peri-insulares izquierdas respecto a variantes amnésicas (Gefen et al., 2012) y los sujetos con variantes conductuales de la EA presentan los mismos hallazgos en zonas frontales (Johnson et al., 1999). Todo esto sugeriría que la variabilidad fenotípica es debida al menos en parte a la variabilidad en la distribución de los cambios patológicos tipo neurofibrilar. Por el contrario, la relación entre la variabilidad fenotípica y la topografía de los depósitos de amiloide es menos clara en estos estudios: las diferencias en los depósitos de amiloide entre grupos eran discretas, corroborando los hallazgos obtenidos *in vivo* que confrontan los resultados de los estudios de PET con marcadores de amiloide y de metabolismo cerebral.

A estos datos, generalmente obtenidos en cohortes relativamente pequeñas, habría que añadir los resultados de trabajos retrospectivos de correlación clínico-patológica en cohortes extensas de sujetos con diagnóstico neuropatológico de EA que han evidenciado fenotipos diferentes desde el punto de vista de la distribución de patología neurofibrilar (Murray et al. 2011). Estos fenotipos patológicos distintos se relacionaban en vida con perfiles clínicos (edad de inicio, sintomatología de debut, velocidad progresión clínica) o radiológicos divergentes (Whitwell et al., 2012). Una vez más, a pesar del elevado número de sujetos analizado, las diferencias en la distribución de patología amiloide entre los diferentes fenotipos clínicos eran más bien discretas (Murray et al., 2011).

En nuestro trabajo las lesiones concomitantes diferentes a las de EA (lesiones vasculares o cuerpos de Lewy) eran, si bien relativamente frecuentes, escasas cuantitativamente en la mayoría de los sujetos y no se correlacionaban de forma significativa con la presentación clínica. Trabajos previos también apuntan que, a diferencia de la enfermedad de inicio tardío, donde es relativamente frecuente encontrar lesiones vasculares o de otras enfermedades

neurodegenerativas, los sujetos más jóvenes suelen tener menos copatología (Savva et al., 2009).

Como es obvio, en todos los trabajos de correlación clínico-patológica la mayoría de los sujetos se analizan en estadios muy avanzados de la enfermedad tanto a nivel clínico como patológico lo que significa una limitación importante para extrapolar los hallazgos a fases iniciales de la enfermedad, que es cuando precisamente se detectan las diferencias clínicas entre la EA típica y atípica. Cabe destacar que las diferencias mencionadas previamente son válidas a nivel grupal, siendo más difícil de demostrar a nivel individual la correlación estricta entre síntomas y topografía lesional.

Por otra parte, varios autores han reportado en diferentes cohortes clínicas la influencia del genotipo *APOE* en el fenotipo clínico de la EA. Así, la presencia de uno o dos alelos ε4 favorecería una presentación amnésica típica mientras que su ausencia se asociaría más frecuentemente a una presentación no-amnésica, o al menos a la presencia de una mayor afectación de otros dominios respeto a la memoria episódica al inicio de la sintomatología (van del Vlies et al., 2007; Snowden et al., 2007; Dickerson et al., 2011; Wolk et al., 2010). Este hallazgo clínico concordaría con estudios de neuroimagen estructural en los que los sujetos con EA en fase de demencia portadores del alelo ε4 tienen mayor atrofia hipocámpica y temporal lateral mientras que en los no portadores se objetiva mayor atrofia en zonas frontoparietales laterales, orbitofrontales y cíngulo posterior (Pievani et al., 2009). La principal crítica que se ha hecho a la mayoría de estos trabajos sería la ausencia de la confirmación biológica del diagnóstico, es decir, los sujetos con presentaciones atípicas podrían tener otras enfermedades neurodegenerativas subyacentes diferentes a la EA, lo que podría explicar las diferencias clínicas y radiológicas encontradas. En nuestra cohorte, no hemos encontrado diferencias en la distribución del alelo de riesgo *APOE* entre los sujetos con presentaciones típicas y atípicas, si bien estos resultados cabe analizarlos con cautela dado el número limitado de sujetos analizados que podría ser insuficiente para identificar posibles diferencias en este sentido.

Una limitación de los estudios basados en cohortes patológicas es el sesgo de remisión, es decir, existe una mayor probabilidad de remisión de las presentaciones atípicas para estudio a un banco de tejidos neurológicos. Por este motivo, podría ocurrir que la frecuencia relativa de presentaciones atípicas en nuestro estudio neuropatológicos podría sobreestimar su prevalencia real. En este sentido, cabría enfatizar que en la cohorte clínica analizada en el

trabajo número 4, aproximadamente el 20% de los sujetos diagnosticados de EAP esporádica con confirmación a nivel del LCR tenían una presentación atípica.

En el trabajo 1 hemos descrito que los sujetos con presentaciones atípicas de la EAP frecuentemente reciben en vida un diagnóstico diferente a la EA: casi el 50% de los casos de presentación no-amnésica no fueron diagnosticados de EA *antemortem*, a diferencia de las presentaciones amnésicas donde el porcentaje de pacientes no diagnosticados clínicamente de EA era muy reducido (4%). Los diagnósticos clínicos alternativos a la EA se encontraban dentro del espectro clínico de la degeneración lobular frontotemporal o de enfermedades psiquiátricas demostrando el importante solapamiento clínico que pueden presentar estas entidades en esta franja de edad. Este hecho se podría explicar en parte por un probable sesgo histórico, ya que muchos de los sujetos evaluados habían sido diagnosticados muchos años atrás, cuando el conocimiento de las presentaciones atípicas de la EA era mucho menor que en la actualidad. Sin embargo, los resultados son concordantes con otras cohortes clínicas o patológicas publicadas en los últimos años sugiriendo que representarían una aproximación razonable a la realidad y en conjunto evidenciarían que las presentaciones atípicas en la EAP son relativamente frecuentes y que únicamente con criterios clínicos es difícil llegar a un diagnóstico precoz y específico, poniendo de relieve la importancia de la utilización de nuevas herramientas biológicas en el proceso diagnóstico. Un diagnóstico clínico correcto es de crucial importancia dado que actualmente sólo la EA dispone de tratamientos específicos; además, el pronóstico, las posibles complicaciones y connotaciones para consejo genético son diferentes entre diferentes enfermedades. Por otra parte, en un futuro, terapias biológicas específicamente diseñadas a influir en aspectos fisiopatológicos concretos de la enfermedad requerirán tener la certeza de la patología subyacente antes de iniciar el tratamiento. Todo ello subraya la necesidad de disponer de biomarcadores específicos de la enfermedad que complementen y mejoren el diagnóstico clínico.

Múltiples trabajos publicados en los últimos años avalan la presencia de un perfil típico de alteración en el LCR en sujetos con EA, caracterizado por una disminución del Aβ42 conjuntamente con un aumento de t-tau y p-tau. Este perfil está ya presente en el momento de la aparición de la sintomatología, incluso en fase de deterioro cognitivo leve y se mantiene a lo largo de toda la fase sintomática (Mattsson et al., 2009). La evidencia existente sobre la utilidad de los marcadores bioquímicos en LCR ha hecho que estos estudios se hayan incluido en los nuevos criterios diagnósticos de la NIA-AA como evidencia del proceso fisiopatológico de la EA *in vivo* (Albert et al., 2011; McKhan et al., 2011). El

trabajo número 4 intenta realizar una aproximación a la "práctica clínica real" analizando de forma prospectiva sujetos con deterioro cognitivo de inicio precoz valorados en la UATC y en los que se ha realizado un estudio de marcadores bioquímicos en LCR..

Los resultados presentados en el trabajo 4 demuestran que los marcadores de LCR presentan una alta sensibilidad y especificidad para el diagnóstico diferencial entre la EAP y controles o sujetos con demencia frontotemporal, mejorando el diagnóstico clínico en fases iniciales y aumentando el grado de probabilidad diagnóstica sobre de la causa subyacente a los síntomas. También hemos evidenciado que una alteración basal de marcadores en LCR en pacientes con DCL de inicio precoz predice la progresión clínica a una fase de demencia tras un seguimiento medio de tres años.

Los valores de sensibilidad y especificidad encontrados en nuestro estudio son robustos y en línea de las publicaciones previas. La mayor especificidad y valor predictivo positivo de estos marcadores bioquímicos en EAP respecto a datos publicados en EAT ya había sido sugerida anteriormente (Bouwman et al., 2009) y se relacionan con una menor proporción de sujetos control con enfermedad preclínica en esta categoría de edad. Una mención especial merece el rendimiento obtenido con índices compuestos, como por ejemplo el ratio Aβ42/p-tau, que muestra sensibilidades y especificidades por encima del 90%. Asociar en un único biomarcador los datos referentes a la amiloidosis y la neurodegeneración presumiblemente aumentaría el rendimiento diagnóstico de la prueba ya que hace más improbable que sujetos con patologías diferentes a la EA presenten esta alteración. Así, en trabajos previos, el ratio Aβ42/p-tau ha demostrado aumentar la sensibilidad y especificidad para discriminar entre sujetos con EA y controles (Fagan et al., 2007; Welge et al., 2009; Fagan et al., 2011) así como aumentar el poder predictivo de progresión clínica a demencia en sujetos con DCL (Parnetti et al., 2012) respecto a valores independientes de Aβ42 o p-tau.

El uso de marcadores de LCR añadido a la información clínica y radiológica en nuestra cohorte clínica ha permitido maximizar la probabilidad de un diagnóstico clínico de EA en un porcentaje relevante de sujetos (en torno al 90%) utilizando los nuevos criterios de DCL y EA (Albert et al., 2011; McKhan et al., 2011). También es importante mencionar que el perfil de biomarcadores en LCR característico de la EA estaba presente tanto en presentaciones típicas como atípicas de la EAP, reforzando la utilidad práctica de la prueba.

En concordancia con publicaciones previas, realizadas principalmente con pacientes con DCL de inicio tardío, encontramos un alto valor predictivo del perfil de biomarcadores en

LCR característico de EA para la conversión a demencia en pacientes con DCL de inicio precoz. Así, en la cohorte analizada todos los sujetos que progresan clínicamente a lo largo del seguimiento y cumplen criterios clínicos de demencia causada por EA tenían en la evaluación basal un perfil de biomarcadores en LCR alterado. Únicamente un paciente con dicho perfil alterado no había desarrollado demencia al cierre del estudio, si bien el seguimiento de este caso era escaso (6 meses). Por el contrario ninguno de los pacientes con un perfil de biomarcadores en LCR normal convirtió a demencia durante el seguimiento.

La principal limitación del uso de biomarcadores en LCR en la actualidad, aparte de la estandarización de los procedimientos analíticos, son la falta de accesibilidad y evaluación de su rendimiento diagnóstico fuera de centros de investigación. Así, hay relativamente poca literatura existente acerca del uso de marcadores en LCR en la práctica clínica habitual. Le Bastard y colaboradores (Le Bastard et al., 2010), en una muestra de deterioro cognitivo de inicio tardío con confirmación patológica, demostraron que los marcadores de LCR habrían conseguido clasificar de forma correcta aproximadamente el 80% de los sujetos con diagnósticos clínicos inciertos. Kester y colaboradores (Kester et al., 2010) evidenciaron que el uso de marcadores de LCR podría aumentar el grado de certeza diagnóstica de los clínicos en aproximadamente un tercio de los casos analizados y podría llevar a un cambio diagnóstico en aproximadamente un 10% de la cohorte. Shooonenboom y colaboradores (Shooonenboom et al., 2012) analizaron una amplia cohorte de sujetos con diferentes diagnósticos degenerativos y no-degenerativos mostrando una alta sensibilidad de los marcadores de LCR (superior al 96%), si bien con baja especificidad. Una de las causas de la baja especificidad en dicho trabajo es la presencia de un número relevante de sujetos mayores de 65 años con diagnóstico clínico de deterioro cognitivo de causa vascular o enfermedad por cuerpos de Lewy y con marcadores de LCR alterados. En este sentido, cabe resaltar que el hecho que las demencias de inicio precoz suelan presentar menos copatología implica un mayor valor predictivo positivo de un marcador alterado en LCR (Schneider et al., 2007; Savva et al., 2009).

Entre las características especiales del estudio de la demencia en esta franja de edad (< 65 años) cabe destacar la alta aceptación por parte de los pacientes de la realización de una prueba invasiva como es una punción lumbar. En este sentido, menos de un 10% de los pacientes a los que se les propuso la realización del estudio lo rechazaron o presentaban contraindicaciones para el procedimiento. Esta buena aceptación de la prueba sería otro dato

que apoyaría la realización del estudio de biomarcadores en LCR como herramienta diagnóstica sistemática en el estudio del deterioro cognitivo de inicio precoz.

La principal limitación en la validación de nuestros resultados es la ausencia de confirmación patológica del diagnóstico, sin embargo el hecho de que el diagnóstico se haya realizado tras un período relativamente largo de seguimiento, basado en criterios clínicos actualizados y apoyado por diferentes biomarcadores (RM, FDG-PET o marcadores genéticos) aumenta la probabilidad de que las diferentes categorías diagnósticas representen de forma correcta la fisiopatología subyacente,

El estudio de marcadores bioquímicos no sólo tiene una utilidad diagnóstica sino que nos permite realizar estudios de aproximación fisiopatológica *in vivo*, dada la correlación reportada entre los niveles de Aβ42 y la carga de β-amiloide cerebral (Tapiola et al., 2009; Seppälä et al., 2012), y entre los niveles de tau y patología neurofibrilar (Shaw et al., 2009). En este sentido, en el trabajo 2 se han comparado los niveles de estos marcadores bioquímicos en pacientes con EAP esporádica y genética causada por mutaciones en el gen de *PSEN1*. El hallazgo más importante en la comparación de los biomarcadores en LCR entre portadores de mutaciones en *PSEN1* y sujetos con EAP esporádica ha sido la presencia de niveles significativamente menores de Aβ42 en portadores de mutaciones respecto a la enfermedad esporádica, sin haberse encontrados diferencias entre los niveles de t-tau y p-tau entre los dos subgrupos. Si existe una correlación directa entre los niveles LCR de Aβ42 y la carga de β-amiloide cerebral, como se ha reportado en trabajos previos (Tapiola et al., 2009; Seppälä et al., 2012), la mayor reducción de los niveles de Aβ42 en portadores de mutaciones sugeriría una mayor carga de β-amiloide en estos sujetos. Este hecho es concordante con trabajos patológicos previos que describen de forma constante un aumento de la carga de Aβ42 en portadores de mutaciones respecto a la enfermedad esporádica (Shepherd et al., 2009). Sin embargo, cabe la posibilidad de que este fenómeno no sea extensible a todas las mutaciones en *PSEN1* u otras alteraciones genéticas causantes de EA ya que se han descrito diferencias en la producción de isoformas de Aβ dependiendo de la posición de la mutación dentro del gen (Portelius et al., 2012).

Por el contrario, no se encontraron diferencias relevantes en los niveles de t-t o de p-tau entre sujetos con EAP esporádica y con mutaciones en *PSEN1* con un grado equiparable de severidad clínica. Según el modelo propuesto por Jack y colaboradores (Jack et al. 2010), en las fases sintomáticas el daño neuronal estaría directamente relacionado con la progresión clínica. Por otra parte, una mayor carga neurofibrilar a nivel neuropatológico se relaciona de

forma positiva con la gravedad de los síntomas (Giannakopoulo et al., 2003; Josephs et al., 2008). Por ello, hay trabajos que proponen la utilización de los niveles de proteína tau en LCR con fines pronósticos (Wallin et al. 2010, Buchhave et al. 2012).

Otro de los marcadores biológicos propuestos para mejorar el diagnóstico clínico de la EA y evaluar la progresión de la enfermedad es la RM estructural. Estudios previos han descrito un patrón característico de alteración en neuroimagen: zonas como la temporal medial, cíngulo posterior, precuneus, el córtex temporal lateral o el giro angular están típicamente afectadas en sujetos con EAT respecto a controles desde fases sintomáticas muy iniciales (Dickerson et al., 2009). En el trabajo 2 realizamos un estudio grupal de neuroimagen estructural en pacientes con EAP con biomarcadores bioquímicos compatibles con EA. Nuestros datos confirman este patrón de pérdida de substancia gris en EAP respecto a controles cognitivamente sanos. Así, tanto los sujetos con enfermedad esporádica como los de causa genética presentan este patrón de extensas zonas de pérdida del grosor cortical respecto a sujetos control que abarca zonas como el córtex cingular posterior, precuneus, córtex parietal inferior y superior, giro supramarginal, hipocampo, giro parahipocampal, giro fusiforme o el córtex occipital. En el análisis de VBM se encuentran resultados parecidos, con grandes áreas de pérdida de sustancia gris tanto corticales como subcorticales respecto a controles. Sin embargo, en la comparación directa de sujetos con EAP esporádica y causada por mutaciones en *PSEN1*, con un nivel similar de severidad clínica, no se objetivaron diferencias significativas en el grado o distribución de la pérdida de substancia gris cortical o subcortical. No podemos descartar, sin embargo, que la falta de significación estadística en alguna de las áreas pueda atribuirse a un efecto del tamaño de la muestra. Cabe mencionar que estos resultados aportan información novedosa ya que la cantidad de estudios previos en este tema concreto es muy escasa.

La ausencia de diferencias en marcadores de neurodegeneración entre enfermedad esporádica y genética, tanto a nivel del LCR como a nivel de neuroimagen, aún con diferentes grados de amiloidosis cerebral, apoyaría las evidencias previas de que la neurodegeneración en la fase de demencia de la EA es, en buena medida, independiente del grado de amiloidosis (Jack et al., 2013). Según el modelo de la cascada amiloide, la etiología sería diferente en el caso de la EA esporádica respecto a genética, con un aumento de producción de β-amiloide en casos genéticos y una disminución del aclaramiento del mismo en casos esporádicos. A pesar de ello, al cabo de décadas de evolución biológica, en la fase presintomática tardía y durante la fase sintomática, se sugiere un *plateau* de β-amiloide con

cambios en marcadores de neurodegeneración relacionados con la aparición y progresión de la clínica tanto en sujetos con enfermedad esporádica como en portadores de mutaciones (Fagan et al., 2009; Bateman et al., 2012; Villemagne et al., 2013), dónde el principal mecanismo patogénico de la progresión clínica no sería ya el β-amiloide, sino probablemente la patología neurofibrilar junto a la inflamación y presencia de lesiones concomitantes. Por ello, a pesar de que los sujetos portadores de mutaciones probablemente tengan una mayor carga de β-amiloide cerebral a lo largo de toda la evolución biológica de la enfermedad, es probable que en fases sintomáticas avanzadas este hecho pierda relevancia.

Los resultados presentados en el trabajo 3, apoyarían también la convergencia biológica de las formas de EAP esporádicas y genéticas, al menos en fases de demencia avanzada. Ambos grupos de enfermos presentan un patrón de expresión génica en el cíngulo posterior caracterizado por una alteración global en vías de señalización (de los cuales la alteración más marcada era a nivel de señalización vía calcio), formación de redes neuronales, vías de potenciación a largo plazo o marcadores de disfunción sináptica respecto a sujetos control, si bien no se encontraron diferencias relevantes entre sujetos con EA precoz esporádica y genética. La principal limitación de este trabajo, es que al estudiar pacientes con una EA en estadio de demencia avanzada no podemos descartar que, a pesar de no encontrar diferencias en las vías biológicas alteradas entre formas esporádicas y formas genéticas, estas existan en fases más precoces de la enfermedad.

Finalmente, si bien en más de un 90% de los casos con EAP no se objetivan alteraciones genéticas que justifiquen la enfermedad, la presencia de historia familiar de EAP es el principal criterio para recomendar la realización de estudios de genes determinantes para descartar una causa genética de la enfermedad, en ausencia de otros marcadores clínicos distintivos. La presencia de una HAD y edad de inicio precoz es altamente sugestiva de mutaciones en genes de la presenilina o *APP*, si bien existe un porcentaje entre 10-20% de pacientes con EAP y HAD en los que no es posible identificar alteraciones en dichos genes (Fortea et al., 2011), sugiriendo la existencia de otras causas genéticas todavía no identificadas. La mutación descrita, L235R, es una nueva mutación en el gen *PSEN1* que se manifiesta con un cuadro clínico y biológico típico con excepción de una rápida progresión clínica. Si bien las diferentes mutaciones en *PSEN1* podrían alterar de forma tanto cuantitativa como cualitativa (Portelius et al., 2010; Portelius et al., 2012) la producción de isoformas amiloidogénicas de la proteína β-amiloide, la diferente agresividad clínica en la fase sintomática de la enfermedad entre diferentes mutaciones es difícilmente explicable

dentro del modelo propuesto por Jack y colaboradores (Jack et al., 2013). Ello nos sugiere que si bien este modelo es válido para explicar la mayor parte de los hallazgos obtenidos en muchos de los estudios clínicos, incluyendo los aquí presentados, hay elementos de la patogenia de la enfermedad que no se encuentran todavía plenamente explicados por ese modelo y que requerirán nuevos abordajes de estudio.

VIII.

CONCLUSIONES

CONCLUSIONES

1. Un tercio de los sujetos estudiados con enfermedad de Alzheimer de inicio precoz esporádica con confirmación neuropatológica tiene una presentación clínica no amnésica. Este hecho comporta que un porcentaje relevante de ellos no reciban el diagnóstico de enfermedad de Alzheimer en vida. El genotipo *APOE* o la presencia de lesiones patológicas concomitantes de forma localizada no parece influenciar la presentación clínica de estos sujetos.

2. Los pacientes con enfermedad de Alzheimer de inicio precoz esporádico presentan un perfil típico de alteración de los biomarcadores en LCR con disminución de Aβ42 y aumento de t-tau y p-tau. La neuroimagen estructural por RM muestra un patrón de pérdida de substancia gris que afecta predominantemente al cíngulo posterior, precuneus, lóbulo temporal lateral, regiones parietales, ínsula, fusiforme, hipocampo y parahipocampo. Respecto a pacientes con enfermedad esporádica, los portadores de mutaciones en el gen *PSEN1* presentan niveles más bajos de Aβ42, sin diferencias en los niveles de t-tau y p-tau o en la severidad de pérdida de substancia gris en la neuroimagen estructural. Estos hallazgos sugieren un mayor depósito amiloideo en fases sintomáticas en portadores de mutaciones que no refleja en marcadores de lesión neuronal, y apoyan la idea de que el grado de neurodegeneración en la fase de demencia de la enfermedad de Alzheimer de inicio precoz es independiente del grado de amiloidosis.

3. Los pacientes con enfermedad de Alzheimer de inicio precoz presentan alteraciones en el perfil de expresión génica en el cingulado posterior de vías biológicas implicadas en la señalización intracelular, interacciones ligando-receptor, formación de redes neuronales y potenciación a largo plazo. No se detectaron diferencias en el patrón de expresión génica entre sujetos con la forma esporádica y genética. Este hecho apoyaría la convergencia de los procesos patológicos en ambos subtipos de la enfermedad, al menos en las fases más tardías del proceso biológico.

4. Los biomarcadores en LCR, especialmente la ratio Aβ42/p-tau, tienen una alta sensibilidad y especificidad, para diferenciar, desde fases iniciales, la enfermedad de Alzheimer de inicio precoz de la degeneración lobular frontotemporal o de cuadros no degenerativos. Además, el uso clínico de marcadores LCR aumenta la probabilidad de un diagnóstico certero de EA en la mayoría de los sujetos, independiente de la presentación clínica y predice con alta fiabilidad la progresión clínica a demencia en sujetos con deterioro cognitivo leve.

5. La mutación L235R es una nueva mutación en el gen *PSEN1* asociada a enfermedad de Alzheimer familiar de inicio precoz y de rápida evolución clínica. La presencia de historia familiar en un caso de enfermedad de Alzheimer de inicio precoz apoya la realización de estudios genéticos para descartar una causa genética.

IX.

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