UNIVERSITAT DE BARCELONA

Utilitat dels marcadors bioquímics i de neuroimatge per al diagnòstic de la malaltia d'Alzheimer d'inici precoç

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INFORME DELS DIRECTORS DE TESI:

El Dr. Albert Lladó, doctor en Medicina per la Universitat de Barcelona i la Dra. Raquel Sánchez del Valle, doctora en Medicina per la Universitat de Barcelona,

CERTIFIQUEN

Que la memòria titulada 'Utilitat dels biomarcadors bioquímics i de neuroimatge per al diagnòstic de la malaltia d'Alzheimer d'inici precoç', presentada per Neus Falgàs Martínez, ha estat realitzada sota la nostra direcció i considerem que reuneix les condicions necessàries per ser defensada davant el Tribunal corresponent per optar al Grau de Doctor.

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ÍNDEX

I. LLISTAT D'ABREVIATURES

$A\beta_{42}$ Isoforma de 42 aminoàcids de la proteïna beta-amiloide

APP Gen de la proteïna precursora d'amiloide

DCL Deteriorament cognitiu lleu

LCR Líquid cefalorraquidi

MA Malaltia d'Alzheimer

MAG Malaltia d'Alzheimer genètica

MAP Malaltia d'Alzheimer d'inici precoç

MAT Malaltia d'Alzheimer d'inici tardà

MMSE Mini-Mental State Examination

MTA Escala d'atròfia medial temporal

Ng Neurogranina

NfL Neurofilaments de cadena lleugera

NIA-AA National Institute of Aging - Alzheimer Association

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

PET Tomografia per emissió de positrons

PET-amiloide PET amb traçador de proteïna amiloide

PET-FBB PET amb traçador de proteïna amiloide 18-F-Florbetaben

PET-FDG PET cerebral amb traçador $_{18}$F-fluorodexoxiglucosa

PICOGEN Programa d'informació i consell genètic per a demències familiars de l'Hospital Clínic de Barcelona.

P-Tau Proteïna Tau fosforilada

PL punció lumbar

PSEN Gen de la presenilina

RM Ressonància magnètica

T-Tau Proteïna tau total

14-3-3 Proteïna 14-3-3

II. INTRODUCCIÓ

INTRODUCCIÓ

1. Epidemiologia, definició i variants clíniques de la malaltia d'Alzheimer d'inici precoç

La malaltia d'Alzheimer (MA) és una malaltia neurodegenerativa que produeix un deteriorament cognitiu progressiu. A nivell neuropatològic, els canvis típics de la malaltia consisteixen en el dipòsit extracel·lular de proteïna amiloide ($A\beta_{42}$) i el dipòsit intracel·lular de proteïna tau hiperfosforil·lada. Aquests canvis poden precedir fins a 15 o 20 anys l'inici dels símptomes, etapa que coneixem com a fase preclínica de la malaltia (Sperling et al. 2011). Progressivament, i a través de mecanismes complexes es produeix la mort neuronal amb la conseqüent atròfia cerebral. A nivell clínic, aquestes alteracions neuropatològiques es tradueixen en l'aparició de símptomes, inicialment en forma de deteriorament cognitiu lleu i finalment, demència.

En base a estudis poblacionals, s'estima que en els darrers anys hi ha hagut un increment dels casos prevalents de demència a nivell mundial. S'ha descrit que aquest augment ha sigut d'un 117% des de l'any 1990 al 2016, essent actualment, la prevalença global de demència d'uns 50 milions de persones. Degut a l'augment de l'esperança de vida i el caràcter crònic propi de la malaltia, es preveu que es mantingui aquest augment dels casos en els propers anys. S'estima que la prevalença es duplicarà cada 20 anys i s'assoliran així, les xifres de 80 milions d'afectes al 2030 i de 152 milions l'any 2050 (World Alzheimer report 2018 - Alzheimer's disease International, CDB 2016 Dementia collaborators, 2019).

Tot i que existeixen diferents malalties que poden causar una demència, la causa més freqüent és la MA, que n'és la responsable d'aproximadament el 76% dels casos. L'edat és el major factor de risc per patir-la. La seva incidència es duplica cada 5 anys a partir dels 65 anys d'edat i per tant, té lloc majoritàriament en fases avançades de la vida (Garré- Olmo et al., 2018). Però tot i així, la MA també la poden patir persones més joves. De forma arbitrària es va establir el llindar de 65 anys per classificar la MA segons l'edat. Així, es va definir la MA d'inici precoç (MAP) com aquella que inicia els símptomes abans dels 65 anys, i la MA d'inici tardà (MAT), com la que inicia els símptomes per sobre d'aquesta edat. A nivell epidemiològic, alguns estudis descriuen

que la MAP té una incidència d'aproximadament 13 casos per 100.000 persones/any i que aquesta conforma un 5-10% dels casos totals de MA (Garré-Olmo et al. 2010; Mercy et al. 2008).

A més de les diferències epidemiològiques, la MAP també té unes característiques clíniques i evolutives diferents a aquells pacients amb MAT. Segons els símptomes que presenti el pacient podem diferenciar-ne dues variants clíniques: la amnèsica i la no amnèsica. La **variant amnèsica o típica**, és aquella en que el problema de memòria és el símptoma predominant des de l'inici de la malaltia. A mesura que la malaltia avança, els dèficits cognitius poden extendre's progressivament a altres àrees cognitives més enllà de la memòria i donar lloc a problemes de llenguatge, viso-espaials i de conducta però el dèficit mnèsic és el primer en aparèixer i el que predomina al llarg del procés de malaltia. En el cas de la MAT aquesta és la variant més freqüent ja que representa un 94% dels casos, sent menys freqüent en la MAP (68%). Per altra banda, la **variant no amnèsica**, consisteix en l'afectació predominant d'una àrea cognitiva que no és la memòria (Koedam et al., 2010). Les formes de presentació no amnèsiques que trobem més freqüentment a la MAP són les alteracions del llenguatge, generalment en forma d'afàsia logopènica (17%), les alteracions viso-espaials i les apràxies (13%) o la disfunció executiva i conductual (2%)(Koedam et al., 2010). A la MAP, la variant no amnèsica és molt més freqüent que en la MAT, ja que pot estar present fins a un 30-40% dels casos.

L'evolució clínica dels pacients amb MAP també és diferent d'aquells amb inici tardà. Tot i la heterogeneïtat en la progressió, els pacients amb MAP poden presentar un curs clínic més agressiu i un declivi més ràpid de les funcions cognitives ja present en els primers anys d'evolució de la malaltia (Wattmo et al., 2017). En estudis post-mortem en pacients amb MAP, també s'han descrit una major càrrega de plaques neurítiques i cabdells neurofibril·lars així com una major pèrdua sinàptica comparat amb aquells pacients amb MAT. Així, aquesta progressió clínica més ràpida podria estar relacionada a una major severitat de canvis neuropatològics característics de la MA subjacents en aquests pacients (Bigio et al., 2002).

Tot i que la MAP és majoritàriament **esporàdica**, es coneix que fins a un 0,5% dels pacients amb MA té un origen **genètic**. En aquests casos, la presència d'una mutació genètica és un factor determinant per patir la malaltia i aquesta s'hereta amb un patró de transmissió autosòmic dominant amb una edat d'inici abans dels 65 anys. Les mutacións més freqüents tenen lloc al gen presenilina 1 (*PSEN1*), seguit del gen de la proteïna precursora de amiloide (*APP*) i el gen presenilina 2 (*PSEN2*). Habitualment, l'inici dels primers símptomes a la MA genètica (MAG) és molt precoç, dels 20 als 50 any, sent la penetrància gairebé completa als 65 anys.

2. Diagnòstic de la malaltia d'Alzheimer

Clàssicament, la MA era considerada una entitat clinico-patològica, és a dir, per al seu diagnòstic era condició necessària complir els criteris sindròmics de demència, i l'estudi anatomopatològic era l'única eina de confirmació diagnòstica. Però en els darrers anys la conceptualització de la MA ha canviat i actualment es considera una entitat clínico-biològica amb un fenotip clínic que varia des de cognició normal (fase preclínica) fins a fases avançades de demència, que es presenta amb símptomes cognitius diversos (variants amnèsica i no amnèsica) i de la que s'en pot fer un diagnòstic mitjançant biomarcadors biològics de la malaltia. Tot i que el diagnòstic de certesa definitiu de MA s'estableix amb l'estudi anatomopatològic, al llarg del temps s'han definit criteris pel seu diagnòstic en vida, que alhora s'han anat modificant degut al desenvolupament de noves eines diagnòstiques. Inicialment, el diagnòstic de MA s'establia únicament en fase de demència, que és la fase de la malaltia en què el dèficits cognitius interfereixen en el desenvolupament de les activitats de la vida diària del pacient, i que per tant, afecta a la seva funcionalitat global (DSM IV- American Psychiatric Association, 2000; ICD-10-World Health Organitzation, 1993). Els primers criteris que definien la demència deguda a MA van ser publicats l'any 1984 pel National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer´s Disease and Related Disorders Association (NINCDS-ARDRA) (McKhann et al., 1984). Aquests permetien establir el diagnòstic de MA en vida basant-se majoritàriament en criteris clínics tot i que incloïen exploracions complementàries dirigides a descartar altres causes de demència:

tomografia computeritzada cerebral (TC), electroencefalograma i anàlisi bàsic del líquid cefaloraquidi (LCR). La normalitat d'aquestes proves era un criteri de suport per al diagnòstic de MA així com també la presència d'atròfia cerebral al TC, de la que no s'indicava cap patró concret. Donada la definició eminentment clínica dels criteris degut a l'absència de biomarcadors específics de la malaltia, la seva precisió diagnòstica era relativament baixa, obtenint-se aproximadament un sensibilitat del 80% i especificitat del 70% (Beatch et al 2012., Knopman et al., 2001).

En les últimes dècades s'han desenvolupat diferents biomarcadors de la malaltia que permeten demostrar *in vivo* els canvis neuropatològics propis de la MA. L'any 1998 es va definir i establir per consens que el biomarcador ideal per al diagnòstic de la MA havia de tenir una sensibilitat major del 80% per distingir MA de controls, una especificitat superior al 80% per distingir MA d'altres demències, així com ser validat per cohorts amb confirmació anatomo-patològica (The Ronald and Nancy Reagan Research Institute of the Alzheimer's Association and the National Institute on Aging Working Group, 1998). La determinació de nivells baixos de $A\beta_{42}$ a LCR i la detecció de plaques d'amiloide mitjançant el PET (tomografia per emissió de positrons) amb traçadors d'amiloide, són biomarcadors capaços de demostrar el dipòsit cerebral de proteïna amiloide. Per altra banda, els nivells elevats de proteïna tau total (T-Tau) i tau fosforilada (P-Tau) a LCR, l'hipometabolisme temporo-parietal en el PET cerebral amb traçador $_{18}$F-fluorodexoxiglucosa (PET-FDG) i l'atròfia d'hipocamp o temporo-parietal a la RM, tradueirien diferents moment del procés de neurodegeneració (Jack et al., 2013). El desenvolupament d'aquests biomarcadors va suposar un canvi important en la definició del diagnòstic de la MA ja que va permetre per primera vegada identificar els canvis biològics de la malaltia en vida, així com establir el diagnòstic de la MA en la seva fase prodròmica, és a dir, en fase de deteriorament cognitiu lleu (DCL). Així, l'any 2011 van ser publicats els nous criteris diagnòstics de MA tant per a DCL com per demència i que són vigents per al seu diagnòstic en l'actualitat (McKhann et al., 2011; Albert et al., 2011). Aquests criteris estratifiquen la certesa del diagnòstic de la MA en probabilitat alta, intermèdia o baixa segons s'hagi pogut demostrar o no la presència de dispòsit d'amiloïde i/o neurodegeneració mitjançant els diferents biomarcadors. Tot i que l'ús dels biomarcadors en els criteris diagnòstics es cenyeixen al marc de fases

simptomàtiques de la malaltia, en context d'investigació, aquests biomarcadors permeten l'estudi i detecció dels canvis biològics de la malaltia inclús en fases preclíniques. De fet, en l'àmbit de la recerca es van desenvolupar recentment un nous criteris de classificació de la MA, que permeten classificar i identificar diferents fases de la malaltia, inclós fases preclíniques (Jack et al., 2018). En aquests criteris, la categorització de les diferents fases es realitza principalment en base a la demostració d'alteracions en biomarcadors de a) dipòsit d'amiloide, mitjançant l'estudi de nivells de $A\beta_{42}$ a LCR i PET-amiloide, i b) dipòsit de tau mitjançant l'estudi de nivells de P-Tau a LCR i PET amb traçadors de proteïna tau. Tot i que aquests són els principals factors classificadors, en una segona línia d'evidència, hi apareix la presència de biomarcadors de c) neurodegeneració, i per tant, considerats menys específics com els nivells de T-Tau, PET-FDG i atròfia a RM cerebral.

3. Biomarcadors diagnòstics a la malaltia d'Alzheimer

Determinació de proteïna amiloide i tau en LCR

L'anàlisi del LCR obtingut mitjançant la punció lumbar permet mesurar els nivells de diferents proteïnes, considerades biomarcadors, que ens proporcionen informació dels canvis neuropatològics al cervell a la MA:

-*Proteïna amiloide ($A\beta_{42}$)*: La disminució de la concentració d'aquesta proteïna en LCR es correlaciona amb la quantitat de dipòsit parenquimatós de proteïna amiloide a nivell cerebral, fet demostrat en estudis anatomopatològics (Seppala et al., 2012).

-*Proteïna tau total (T-Tau) i tau fosporilada (P-Tau):* L'augment de la concentració d'aquestes proteïnes a LCR tradueirien la presència de dany neuronal, que provoca l'alliberament de la proteïna tau a l'espai extracel·lular. Tot i que ambdós es consideren biomarcadors de neurodegeneració de la MA, en els darrers anys s'ha suggerit una major especificitat de P-Tau envers T-Tau. Estudis anatomopatològics també han demostrat una bona correlació entre els nivells de P-Tau i T-Tau i els dipòsits cerebral de tau en forma de cabdells neurofibril·lars (Seppala et al., 2012).

L'anàlisi de LCR en el diagnòstic de la MA ha demostrat tenir una bona precisió diagnòstica a l'hora de distingir pacients de controls obtenint sensibilitat i especificitats

per sobre del 80%, complint per tant els criteris establerts prèviament per ser considerat biomarcador de la MA (Mattsson et al., 2017; Seeburger et al., 2015).

PET amb traçador de proteïna amiloide

El PET, mitjançant l'administració de diversos traçadors de proteïna amiloide (florbetapir, florbetaben o flutemetamol) pot detectar *in vivo* la distribució anatòmica de les plaques d'amiloide al cervell. Diversos estudis han demostrat una molt bona correlació entre la positivitat del PET-amiloide i la disminució de $A\beta_{42}$ a LCR així com la distribució de captació del PET-amiloide i el dipòsit parenquimatós de la proteïna en estudis anatomopatològics (Chiotis et al., 2017; Mattsson et al. 2014; Landau et al., 2013). Pel que fa a la seva precisió diagnòstica, aquesta ha mostrat ser superior al 85% en termes de sensibilitat i especificitat en diversos estudis (Chiotis et al., 2017; Yeo et al., 2015; Sabri et al., 2015).

PET cerebral amb traçador $_{18}$F-fluorodexoxiglucosa

El PET-FDG permet saber el grau d'activitat sinàptica mitjançant la mesura del metabolisme de glucosa. El patró característic de la MA és l'hipometabolisme temporo-parietal bilateral encara que pot haver-hi també cert hipometabolisme frontal. La sensibilitat diagnòstica d'aquesta prova ha demostrat ser bona, per sobre del 80% en diversos estudis, però la especificitat és subóptima, entre el 60 i el 75% (Garibotto et al., 2017, Jagust et al. 2017).

Ressonància magnètica cerebral

Es una tècnica disponible i econòmica que permet l'avaluació de la presència d'atròfia de diferents estructures cerebrals. L'hipocamp és una de les regions que s'afecten de forma més precoç a la MA i la seva atròfia s'ha inclòs en els criteris com a un dels biomarcadors diagnòstics. L'atròfia d'hipocamp pot avaluar-se mitjançant una escala visual o bé,

quantificant el volum d'hipocamp. Pel que fa a l'avaluació visual, s'utilitza l'escala de Scheltens que classifica el grau d'atròfia d'hipocamp en cinc categories (0-4). Posteriorment, els valors assignats a l'hipocamp dret i l'esquerre es promitjen donant lloc a l'escala de d'atròfia medial temporal (MTA). Es va establir que el punt de tall considerat patològic per a subjectes menors de 75 anys seria el MTA≥1.5 (Van de Pol et al., 2014). Malgrat s'estan desenvolupant tècniques de mesura semiautomàtica per millorar la detecció de l'atròfia d'hipocamp, el seu processat és complex i dificulta la seva integració a la pràctica clínica. En canvi, la rapidesa i fàcil aplicació de l'avaluació visual fa que sigui molt més utilitzada. Diversos estudis han avaluat la precisió diagnòstica d'ambdues tècniques d'atròfia d'hipocamp a la MAT; aquestes han mostrat certa variabilitat segons l'estudi però en general obtenen bones sensibilitats (80-90%) per discriminar MA de controls però especificitats més baixes (<70%) al discriminar MA d'altres demències (Kate et al., 2017; Harper et al., 2016). En canvi, en el cas de la MAP, el seu rendiment diagnòstic no ha estat ben avaluat. Donat que són pacients més joves i tenen una major freqüència de presentacions no amnèsiques és esperable que tinguin menys atròfia d'hipocamp i que aquesta sigui indetectable si no s'apliquen tècniques de mesura semiautomàtica, així com major atròfia en altres àrees com ara atròfia parietal. La majoria d'estudis en què s'avalua el rendiment diagnòstic de l'atròfia d'hipocamp, consisteixen en cohorts sense confirmació biològica de la malaltia o no estan dirigides exclusivament a MAP.

Estudi genètic

En aquells pacients amb MAP amb un patró d'herència autosòmica dominant de la malaltia també d'inici precoç es recomana l'estudi genètic. La detecció d'una mutació patogènica als gens *PSEN1, PSEN2* o *APP* permet establir per ella mateixa el diagnòstic de la malaltia en un pacient simptomàtic, així com donar consell genètic als seus familiars, oferint la possibilitat que els que ho desitgin poguin conèixer el seu estat genètic tot i no presentar simptomatologia. Tot i que la presència de la mutació és suficient per emetre el diagnòstic de MAG, habitualment es completa l'estudi amb altres biomarcadors bioquímics i de neuroimatge, per tal de poder caracteritzar millor la

malaltia. A l'Hospital Clínic de Barcelona, des del l'any 2002 es duu a terme el Programa d'informació i consell genètic per a demències familiars (PICOGEN) (Fortea et al. 2011), programa que permet tant l'assessorament als pacients portadors de la mutació i les seves famílies, com l'estudi de la trajectòria de diferents biomarcadors biològics en diferents estadis de la malaltia, inclús en fases preclíniques (Bateman et al., 2011, Fleisher et al., 2012). En aquest sentit, hi ha diversos estudis han utilitzat el PET-amiloide amb traçadors florbetapir i *11C-Pittsburgh compound B* per definir diferents patrons de captació en aquests pacients i han demostrat dipòsit d'amiloide fins a 15 anys abans de l'inici del símptomes, especialment a nivell estriatal (Benzinger et al., 2013). En canvi, no hi ha estudis publicats que avaluin la seguretat i els patrons de captació amb el PET-FBB a la MAG.

4. Importància del diagnòstic precoç

El diagnòstic de la MAP sovint és complex. Per una banda, l'aparició dels símptomes a edats precoces de la vida i les presentacions clíniques atípiques poden dificultar la identificació del problema tant per part de pacients i famílies, com per professionals sanitaris, fet que comporta un retard a la consulta mèdica inicial i un retard a la derivació a unitats especialitzades. Per altra banda, la major presència de variants atípiques de la malaltia fa que hi hagi un major solapament clínic amb altres patologies. Aquest solapament té lloc tant amb patologies neurodegeneratives com la demència frontotemporal (DFT), que és la segona causa deteriorament cognitiu d'inici precoç, com amb trastorns no neurodegeneratius com ara els trastorns de l'estat d'ànim. Aquests factors condueixen a una major discrepància entre el diagnòstic clínic i l'anatomopatològic en pacients amb MAP, especialment en fases de DCL i demència lleu (Balasa et al., 2011; Mendez et al., 2006).

Actualment, l'ús dels nous criteris diagnòstics i la possibilitat d'utilitzar diversos biomarcadors (bioquímics, de neuroimatge i genètics) afegeixen evidència del procés fisiopatològic i milloren la certesa diagnòstica. Això permet que el diagnòstic sigui més acurat fins i tot en fases inicials de la malaltia. Fer un diagnòstic precoç i acurat és especialment important en aquests pacients. La MAP té un impacte personal, social,

familiar, legal i econòmic rellevant ja que aquest grup de pacients pertanyen a una franja d'edat en què en general estan laboralment actius i tenen responsabilitats cap a fills joves. El diagnòstic precoç en aquests casos permetrà que la persona afecta pugui tenir el diagnòstic en una fase en la que encara sigui capaç de decidir sobre el seu futur, planificar els recursos socials, iniciar el programa de tractament farmacològic i no farmacològic (estimulació cognitiva), així com la possibilitat de participar en assaigs clínics amb possibles fàrmacs modificadors de l'evolució de la malaltia, que es troben actualment en fase de desenvolupament. Tot i que els biomarcadors han demostrat ser útils i necessaris per a realitzar un diagnòstic acurat i precoç, no hi ha estudis que de forma global hagin fet balanç de quin rendiment diagnòstic o de quin valor afegit proporcionen cada un d'ells al procés diagnòstic en el cas concret de la MAP. De la mateixa manera, tampoc s'ha avaluat el grau d'invasivitat, entès com la tolerabilitat de les proves realitzades (RM cerebral, punció lumbar, PET-amiloide, PET-FDG) per part dels pacients. Per tant, està per determinar quin biomarcador o conjunt de biomarcadors són millors per al diagnòstic de la MAP, tenint en compte ambdós factors, que siguin útils per al clínic en el procés diagnòstic i que siguin alhora ben tolerats pel pacients, per tal de poder així, optimitzar la seva aplicació.

5. Recerca d'altres biomarcadors

Més enllà dels biomarcadors inclosos en els criteris diagnòstics de la MA, a nivell de recerca existeixen altres biomarcadors dels que se n'està avaluant la seva capacitat diagnòstica i/o pronòstica a la MA. Hi ha estudis previs que han descrit el perfil de diversos nous biomarcadors tant a LCR com a nivell de neuroimatge a la MA en cohorts de MAT però no hi ha estudis que ho avaluin en MAP. Alguns d'aquests biomarcadors són el següents:

<u>Biomarcadors a LCR</u>

-*Neurofilament de cadena lleugera (NfL):* El procés de neurodegeneració condueix a la destrucció del citoesquelet neuronal, i per tant, l'alliberament de proteïnes estructurals

com els NfL, que són d'origen axonal. Els seus nivells són detectables a LCR i la seva elevació ha sigut detectada tant en demències neurodegeneratives (DFT i MA) com en altres patologices neurològiques (esclerosi lateral amiotròfica, l'esclerosis múltiple) (Gaiana et al., 2017; Pijnenburg et al., 2007). S'ha proposat com un biomarcador de neurodegenereació inespecífica, i són necessaris més estudis per conèixer millor la seva capacitat tant diagnòstica com pronòstica en la MAP.

-*Neurogranina (Ng):* És una proteïna d'origen dendrític que reflexa la degeneració sinàptica. La seva elevació a LCR s'ha proposat com un marcador específic de la MA donat que el seu increment no s'ha trobat a altres patologies neurodegeneratives (Portelius et al. 2018).

-*Proteïna 14-3-3:* Aquesta proteïna també reflexa dany neuronal sinàptic. Ha estat ampliament estudiada en la malaltia de Creutzfeldt-Jakob considerant-se'n el seu augment a LCR un biomarcador d'aquesta malaltia (Schmitz et al., 2016). Tot i així, aquest és inespecific i per exemple també s'ha descrit la seva elevació en LCR de pacients amb MA (McFerrin et al., 2017).

Biomarcadors de neuroimatge

-*Alteracions estructurals de subtància blanca a RM:* L'estudi de les alteracions a subtància blanca no s'inclou en els criteris diagnòstics de la MA. Tot i així, més enllà de l'extensa bibliografia publicada en relació als patrons d'atròfia cortical a subtància gris, hi ha també diversos estudis que han descrit alteracions de la conectivitat estructural a subtància blanca a la MA. Aquests canvis han estat correlacionats amb els biomarcadors clàssics de la MA ($A\beta_{42}$, tau) tant en estadis simptomàtics de la malaltia com en estadis preclínics (Canu et al., 2017; Pereira et al., 2017, Sala-Llonch et al., 2015). Tot i així, la relació que tenen els canvis estructurals cerebrals observats en pacients amb MA amb la resta de biomarcadors actualment en recerca (NfL, Ng, 14-3-3) no ha estat ben definits.

III. HIPÒTESIS

HIPÒTESIS

1. L'impacte clínic de l'ús dels biomarcadors en pacients amb una alteració cognitiva d'inici precoç serà rellevant.
   a) L'aplicació clínica dels diferents biomarcadors podria comportar un canvi de diagnòstic i/o de tractament fins a un 20% dels pacients.
   b) Els biomarcadors de dipòsit de proteïna amiloide (PET-amiloide i $A\beta_{42}$ a LCR) proporcionarien un augment de certesa diagnòstica per al diagnòstic de pacients amb MAP, la qual seria similar entre ells i superior als biomarcadors de neurodegeneració. Els diferents biomarcadors de neurodegeneració (T-Tau i P-Tau a LCR, PET-FDG, atròfia d'hipocamp a la RM) podrien proporcionar una confiança diagnòstica diferent entre ells al ser indicatius de diferents processos fisiopatològics.
   c) Les diferents proves realitzades per obtenir els biomarcadors diagnòstics (punció lumbar, PET-FDG, PET-amiloïd, RM cerebral) seran majoritàriament ben tolerades, si bé podrien existir algunes diferències entre elles.

2. La mesura de l'atròfia d'hipocamp a la RM cerebral mitjançant una tècnica semiautomàtica podria millorar el rendiment diagnòstic d'aquest biomarcador en el diagnòstic de pacients amb MAP, respecte a la seva avaluació mitjançant l'escala visual MTA.

3. L'avaluació de l'atròfia a la RM cerebral de diferents regions cerebrals mitjançant diferents escales visuals (orbito-frontal, cingulat anterior, fronto-insular, temporal anterior, posterior) podria ser més útil i tenir un major rendiment diagnòstic que l'escala d'atròfia d'hipocamp pel diagnòstic de pacients amb MAP, especialment en pacients amb presentació no amnèsica.

4. La pèrdua de substància gris i substància blanca en àrees típiques de MAP es relacionarà amb l'alteració dels biomarcadors a LCR típics d'aquesta malaltia ($A\beta_{42}$ i T-Tau) però també amb altres biomarcadors de dany cerebral com NfL, Ng i 14-3-3.

5. L'ús de PET-amiloide amb florbetaben en subjectes portadors de mutacions en gens causants de MAG (*PSEN1*), tant asimptomàtics com simptomàtics, serà segur i permetrà identificar patrons regionals i temporals de dipòsit de proteïna amiloide al llarg de la malaltia.

IV. OBJECTIUS

OBJECTIUS

1. Avaluar l'impacte clínic de l'ús de diferents biomarcadors (biomarcadors a LCR, PET-amiloïde, PET-FDG, atròfia d'hipocamp a la RM cerebral) en el diagnòstic de pacients amb MAP en termes de a) canvi de diagnòstic i tractament, b) grau de confiança diagnòstica aportada per cada biomarcador i c) tolerabilitat de les proves.

2. Determinar i comparar el rendiment diagnòstic en pacients amb MAP de l'atròfia de l'hipocamp en RM cerebral mesurada mitjançant dues tècniques: l'escala visual d'atròfia hipocampal (MTA) i la volumetria semiautomàtica de l'hipocamp.

3. Determinar el rendiment diagnòstic en pacients amb MAP de diferents escales visuals que avaluen diferents àrees d'atròfia cerebral (escales orbito-frontal, cingulat anterior, fronto-insular, temporal anterior, posterior). Comparar el rendiment diagnòstic d'aquestes escales amb el de l'escala MTA, tant en pacients que presenten la variant amnèsica com no amnèsica.

4. Establir la relació entre la pèrdua de substància gris i substància blanca característica de pacients amb MAP amb diversos biomarcadors a LCR: $A\beta_{42}$, T-Tau, NfL, Ng, 14-3-3.

5. Determinar la seguretat i eficàcia del PET-amiloide amb florbetaben en subjectes portadors de mutacions en *PSEN1*, simptomàtics i asimptomàtics, i avaluar els patrons de dipòsit regional de proteïna amiloide al llarg de la malaltia.

V. MATERIAL I MÈTODES

MATERIAL I MÈTODES

Els aspectes metodològics utilitzats en la present tesi doctoral es detallen en l'apartat corresponent a cada treball. Tot seguit es descriuen breument els criteris de selecció dels pacients estudiats i les principals tècniques utilitzades.

1. Pacients

Els subjectes seleccionats per aquest treball van ser pacients visitats a les consultes externes de la Unitat d'Alzheimer i altres trastorns cognitius del Servei de Neurologia de l'Hospital Clínic de Barcelona, van ser els següents:

a) MAP: Pacients menors de 65 anys que complien criteris clínics de DCL i demència lleu degut a MA (Albert et al., 2011; McKhann et al., 2011).

b) MAT: Pacients majors de 65 anys que complien criteris de DCL i demència lleu per MA (Albert et al., 2011; McKhann et al., 2011)

c) MAG: Pacients menors de 65 anys complien criteris de DCL i demència lleu per MA (Albert et al., 2011; McKhann et al., 2011) i que fóssin portadors d'una mutació patològica al gen de la *PSEN1*.

d) DFT: Pacients menors de 65 anys que complien criteris diagnòstics de diferents variants de DFT (variant conductual, afàsia primària progressiva semàntica o no fluent (Rascovsky K et al., 2011; Gorno-Tempini ML et al., 2011).

e) DCL de causa no neurodegenerativa: Subjectes menors de 65 anys que complien criteris de DCL amb biomarcadors a LCR negatius.

f) Controls sans: Es van seleccionar tant un grup de subjectes de <65 anys com >65 sense alteracions cognitives (test neuropsicològic normal) amb estudi de biomarcadors a LCR negatius.

Tots els pacients que han participat van signar un consentiment informat. Aquest estudi ha sigut aprovat per el Comité de ètic de l'Hospital Clínic de Barcelona.

2. Mètodes

Les principals tècniques utilitzades per al desenvolupament d'aquesta tesi han sigut les següents:

a) Anàlisi de biomarcadors a LCR: Es va realitzar l'extració de 10mL de LCR mitjançant punció lumbar per a l'anàlisi dels nivells dels diferents biomarcadors. Aquests es van analitzar mitjançant tècnica ELISA amb els kits comercials disponibles per a cada biomarcador: $A\beta_{42,}$ T-Tau i P-Tau (INNOTEST, Fujirebio-Ghent, Belgium), NfL (IBL International, Hamburg, Germany), 14-3-3 (CircuLex, MBL International Corporation, Woburn, MA, USA) al Laboratori de la Unitat d'Alzheimer d'Hospital Clínic-IDIBAPS (Cellex). La Ng (in-house ELISA amb un anticòs monoclonal Ng7) es va analitzar al Clinical Neurochemistry Laboratory, Mölndal.

b) RM cerebral: Totes les imatges es van adquirir en un únic aparell de RM 3.0 Tesla Siemens Magnetom Trio (Erlangen, Germany) del Centre de Diagnòstic per la imatge del Servei de Radiologia de l'Hospital Clínic de Barcelona-plataforma IDIBAPS. Es van utilitzar seqüències MPRAGE (TR=2300 mseg; TE=2,98 mseg; 256 x 256 matrix) i Diffusion Weighted echo-planar imaging (TR = 7600 ms, TE = 89 ms, 122 x 122 matrix.

-*Avaluació d'escales d'atròfia visual*: L'avaluació de les escales visuals (MTA, cingulat anterior, frontoinsular, orbitofrontal, temporal anterior, atròfia posterior) es va dur a terme per dos avaluadors cecs al diagnòstic del pacient.

-*Avaluació quantitativa del volum de l'hipocamp*: Les seqüències T1 van ser processades amb FreeSurfer v5.3 a la plataforma neuGRID (www.neugrid4you.eu) per obtenir el volum hipocampal amb el pipeline nG+FreeSurfer+5.3.0+Diagnostic+v05.xml.

-*Anàlisi del gruix cortical (CTh-Cortical Thickness)*: El processat per obtenir el gruix cortical es va dur a terme ambFreeSurfer v6.0.0 (https://surfer.nmr.mgh.harvard.edu/).

-*Anàlisi de substància blanca:* El processat per obtenir els indexs de integritat de subtància blanca es va realitzar amb FSL v5.0.11.

c) PET-amiloide*:* Després de l'administració del traçador (florbetaben, fluetemetamol o florbetapir) es van adquirir les imatges amb PET molecular (molecular computed tomography PET, Siemens).

-*Avaluació visual:* Es va realizar per radiòlegs experts segons el descrit a les guies.

-*Avaluació quantitativa:* Es van obtenir les ratios de captació de traçador per cada regió (standardized uptake value ratio-SUVR) i posteriorment es van analitzar amb Freesurfer.

c) PET-FDG*:* Després de l'administració del traçador 18-flurordesoxiglucosa es van adquirir les imatges amb PET molecular (molecular computed tomography PET, Siemens). L'avaluació visual es va realizar per radiòlegs experts segons el descrit a les guies.

d) Qüestionari de confiança diagnòstica: Mitjançant un qüestionari el neuròleg indicava, per cada pacient, el diagnòstic de sospita inicial, el diagnòstic final després dels biomarcadors, la confiança diagnòstica proporcionada per cada biomarcador, així com els canvis de tractament.

e) Qüestionari d'invasivitat: A través d'un qüestionari els pacients informaven del grau de tolerabilitat percebuda per cadascuna de les proves realitzades (punció lumbar, PET-amiloide, PET-FDG i RM cerebral) mitjançant escala analògica.

VI. RESULTATS

Treball número 1:

Clinical applicability of diagnostic biomarkers in early onset cognitive impairment.

Falgàs N, Tort-Merino A, Balasa M, Borrego-Écija S, Castellví M, Olives J, Bosch B, Férnandez-Villullas G, Antonell A, Augé JM, Lomeña F, Perissinotti A, Bargalló N, Sánchez-Valle R, Lladó A

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ORIGINAL ARTICLE

Clinical applicability of diagnostic biomarkers in early-onset cognitive impairment

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Background and purpose: Several diagnostic biomarkers are currently available for clinical use in early-onset cognitive impairment. The decision on which biomarker is used in each patient depends on several factors such as its predictive value or tolerability.

Methods: There were a total of 40 subjects with early-onset cognitive complaints (<65 years of age): 26 with Alzheimer's disease (AD), five with frontotemporal dementia and nine with diagnostic suspicion of non-neurodegenerative disorder. Clinical and neuropsychological evaluation, lumbar puncture for cerebrospinal fluid (CSF) AD core biochemical marker determination, medial temporal atrophy evaluation on magnetic resonance imaging, amyloid-positron emission tomography (PET) and ^{18}F-fluorodeoxyglucose-PET were performed. Neurologists provided pre- and post-biomarker diagnosis, together with diagnostic confidence and clinical/therapeutic management. Patients scored the tolerability of each procedure.

Results: Cerebrospinal fluid biomarkers and amyloid-PET increased diagnostic confidence in AD (77.4%–86.2% after CSF, 92.4% after amyloid-PET, $P < 0.01$) and non-neurodegenerative conditions (53.6%–75% after CSF, 95% after amyloid-PET, $P < 0.05$). Biomarker results led to diagnostic (32.5%) and treatment (32.5%) changes. All tests were well tolerated.

Conclusions: Biomarker procedures are well tolerated and have an important diagnostic/therapeutic impact on early-onset cognitive impairment.

Introduction

Early-onset cognitive impairment diagnosis leads to diagnostic difficulties due to the frequent overlap between dementias such as Alzheimer's disease (AD) and frontotemporal dementia (FTD) or non-neurodegenerative disorders [1]. This diagnostic complexity frequently entails greater rates of misdiagnosis and diagnostic delay, especially in mild cognitive impairment (MCI) and mild dementia stages [2]. In order to increase the diagnostic accuracy, different biomarkers have been developed and included in both AD and FTD diagnostic criteria [3–6]. They have been classified into amyloid deposition biomarkers [amyloid-β_{42} ($A\beta_{42}$) level determination on cerebrospinal fluid (CSF) and amyloid-positron emission tomography (PET)] and neurodegeneration biomarkers [tau determination on CSF, medial temporal atrophy (MTA) visual assessment on magnetic resonance imaging (MRI) and ^{18}F-fluorodeoxyglucose (FDG)-PET] [3,4,7]. Currently, although the use of these biomarkers aims to improve

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their diagnostic accuracy and efforts to determine their clinical validity are being made, their impact in the diagnostic and therapeutic approach in prodromal/initial stages in early-onset patients is still uncertain [8,9]. In addition, deciding which diagnostic procedures are used should be a shared decision involving not only clinicians but also patients and caregivers [10]. Therefore, the individualized election of biomarkers should take into account the added diagnostic confidence of each biomarker and the patient's tolerance of procedures. We aimed to describe the diagnostic implications of using diagnostic biomarkers on clinical practice in early cognitive impairment to determine their added diagnostic confidence and the patient's tolerance of the procedures.

Methods

We included 40 subjects who were referred to the Alzheimer's Disease and Other Cognitive Disorders Unit at the Hospital Clínic Barcelona for early-onset cognitive complaints (<65 years of age and Mini-Mental State Examination score $\geq$ 18). The study was approved by the Hospital Clinic Barcelona Ethics Committee and all participants gave written informed consent. Neurological and neuropsychological evaluations to assess cognitive and functional performance were performed. The treating neurologist (N.F., R.S.-V., M.B., S.B., A.L.) determined the initial diagnosis based only on clinical features as well as its diagnostic confidence (range, 0%–100%). Patients with initial diagnosis of AD, behavioral variant of FTD (bvFTD), non-fluent variant of primary progressive aphasia (nfvPPA), semantic variant of primary progressive aphasia (svPPA), dementia with Lewy bodies (DLB) or non-degenerative condition (i.e. anxiety, depression, bipolar disorder, fibromyalgia, chronic fatigue syndrome) were included [3–6,11]. Patients underwent the following procedures.

a) Magnetic resonance imaging to MTA visual assessment. High-resolution sagittal T1-weighted MRI images were acquired on a 3-T scan (Magnetom Trio, Siemens, Erlangen, Germany) at the MRI Core Facility/IDIBAPS platform. An expert radiologist who specialized in dementia neuroimaging (N.B.) visually assessed the right/left hippocampus on T1-coronal images to score MTA according Scheltens' scale [7,12].

b) Lumbar puncture (LP). Levels of $A\beta_{42}$, total-tau and phosphorylated-tau were measured using commercial sandwich enzyme-linked immunosorbent assay kits (Fujirebio, Gent, Belgium) [13].

c) ^{18}F-fluorodeoxyglucose-positron emission tomography. FDG-PET was performed according to current guidelines and experienced nuclear medicine physicians (F.L., A.P.) evaluated whether the pattern of brain hypometabolism was consistent with AD (temporoparietal), FTD (frontotemporal), svPPA (left posterior frontoinsular), nfvPPA (anterior temporal) or non-specific.

d) Amyloid-PET. Florbetapir (n = 29), flutemetamol (n = 10) or florbetaben (n = 1) ligands were used for PET acquisition. Nuclear medicine physicians (F.L., A.P.) evaluated the visual uptake pattern in accordance with the current guidelines. Both FDG-PET and amyloid-PET images were acquired in a Biograph molecular computed tomography PET (mCT PET) computed tomography scanner (Siemens).

After the procedures, neurologists updated the diagnosis based not only on clinical features but also taking into account biomarker results [3–6,11]. AD diagnosis included MCI due to AD and dementia due to AD [3,4]. Non-degenerative condition diagnosis was established in those with compatible clinical features and non-altered specific biomarkers. Neurologists also scored diagnostic confidence provided by each biomarker. Changes in treatment with cholinesterase inhibitors were also reported and defined as follows: (i) starting the treatment in patients with AD in whom it was not started before as the initial suspicion was a non-AD disorder or (ii) not starting or withdrawing the treatment in those patients with initial suspicion of AD that biomarkers finally ruled out. Participants scored the discomfort they experienced during each procedure (range, 0–10) categorized as: no discomfort (0–1), mild (2–4), moderate (5–7) and severe (8–10) discomfort. The reason for the discomfort and willingness to repeat the procedure were categorized as: 'Yes, sure', 'Probably yes', 'Probably no' and 'No, never, it was too annoying'.

Statistical analysis

Statistical analysis was conducted using Stata/IC 14.2. (College Station, Texas, USA) Differences in demographic data, biomarker results and tolerability were assessed by t-test for quantitative data and χ^2 test for categorical data. Change in diagnostic confidence pre- and post-biomarkers was assessed by paired-sample t-tests.

Results

Demographics and clinical data

Demographic data are shown in Table 1. The mean age of participants was 58.9 (SD 3.9) years, mean age of symptom onset was 56.4 (SD 5.2) years and 52.5%

Table 1 Demographic and clinical data according to the initial diagnosis

Initial diagnosis	AD (n = 26)	FTD (n = 6)	NN (n = 9)
Age (years)	59.3 ± 3.5	58.8 ± 4.2	57.8 ± 0.5
Sex (female)	53.8	20	44.4
Age at symptom onset (years)	56.8 ± 5.3	56.4 ± 3.7	54.9 ± 6.2
MMSE score	21.8 ± 3.4*	26.2 ± 1.5	26.5 ± 1.1
Apoε4[a]	39.1	40	50
Symptom at onset			
Memory	61.5	0	77.8
Language	11.5	20	0
Visuospatial	15.38	0	11.1
Executive-behavioral	11.54	80	11.1

AD, Alzheimer's disease; FTD, frontotemporal dementia; MMSE, Mini-Mental State Examination; NN, non-degenerative condition; [a]Patients with at least one allele of apolipoprotein E type 4 allele (Apoε4); *$P < 0.05$. Data are given as mean ± SD and %.

were male with no statistical differences between groups. Initial diagnosis was AD in 26 subjects (65%) (12 MCI-AD and 14 mild dementia), FTD in five subjects (12.5%) and non-degenerative condition in nine subjects (22.5%). The Mini-Mental State Examination score was lower in the AD group (21.8, $P < 0.05$).

The final diagnosis was AD in 20 subjects (50%) (7 MCI-AD and 13 mild dementia), 19 of them with high likelihood and one with intermediate likelihood of AD pathophysiological process, according to National Institute on Aging-Alzheimer's Association criteria [3,4]. Ten patients (25%) were diagnosed with FTD, six with probable bvFTD diagnosis, one with definitive bvFTD diagnosis, two with imaging-supported svPPA diagnosis and one with imaging-supported nfvPPA diagnosis [5,11]. Nine patients (22.5%) were diagnosed with non-degenerative disorder and one patient (2.5%) with DLB [6].

Biomarker results

In 25% of participants, the LP was not performed due to technical difficulties or patient consent withdrawal. MRI was not performed due to claustrophobia in 5% of subjects. All subjects underwent amyloid-PET and FDG-PET, although 5% of FDG-PET scans were not interpretable due to hyperglycemia.

Distribution of biomarker results according to the initial diagnosis is shown in Table 2. CSF biomarkers showed combined decreased $A\beta_{42}$ and increased tau levels in 55% of patients with initial diagnosis of AD, 33% of those with suspicion of non-neurodegenerative disorder and none of those with suspected FTD. Amyloid-PET was negative for all patients with suspected FTD and positive for 65% of those with suspicion of AD and 33% of those with a suspected non-neurodegenerative condition. MTA score ≥ 1.5 was present in all suspected diagnoses of FTD, 48% of AD and 11% of non-neurodegenerative conditions. The 45% of subjects with suspected non-neurodegenerative disorder had normal FDG-PET but 33% showed an AD hypometabolism pattern and 22 were non-specific. An AD hypometabolism pattern was found in 69% of those with a pre-biomarker clinical diagnosis of AD. In the case of patients with suspected FTD, 80% had an FTD hypometabolism pattern and 20% had an AD pattern. Good concordance with amyloid results and decreased $A\beta_{42}$ CSF were found.

Changes in clinical diagnosis

Biomarker results caused a diagnostic change in one-third (13) of the participants (Fig. 1). Nine of the patients with initial AD diagnosis (34.6%) had a changed diagnosis after biomarkers: five patients were diagnosed with FTD variants (three bvFTD, one nfvAPP and one svPPA) and four with a non-neurodegenerative disorder. Furthermore, there was a diagnostic change in four patients with initial diagnosis of non-degenerative disorder (44.4%) (three were diagnosed with AD and one with DLB). Biomarker results confirmed FTD in all patients with initial

Table 2 Biomarker results according to initial diagnosis

Initial diagnosis	CSF				MTA score	FDG-PET				Amyloid-PET	
	Abnormal[a]	Normal[b]	$A\beta_{42}$[c]	Tau[d]	≥1.5	AD pattern[e]	FTD pattern[f]	Normal[g]	Non-specific[h]	Positive	Negative
AD	55	30	10	5	48	69	11.5	11.5	8	65	35
FTD	0	100	0	0	100	20	80	0	0	0	100
NN	33	50	17	0	11	33	0	45	22	33	67

AD, Alzheimer's disease; CSF, cerebrospinal fluid; FDG, ^{18}F-fluorodeoxyglucose; FTD, frontotemporal dementia; MTA, medial temporal atrophy; NN, non-degenerative condition; PET, positron emission tomography; [a]Decreased amyloid-β_{42} ($A\beta_{42}$) and increased total-tau (t-tau) and phosphorylated-tau (p-tau) levels; [b]Normal $A\beta_{42}$ and t-tau and p-tau levels; [c]Only decreased $A\beta_{42}$ with normal t-tau and p-tau levels; [d]Only elevated t-tau and p-tau with normal $A\beta_{42}$ levels; [e]Temporoparietal hypometabolism; [f]Frontotemporal, left posterior frontoinsular or anterior temporal hypometabolism; [g]No hypometabolism; [h]Non-specific or non-interpretable due to hypoglycemia. Data are given as %.

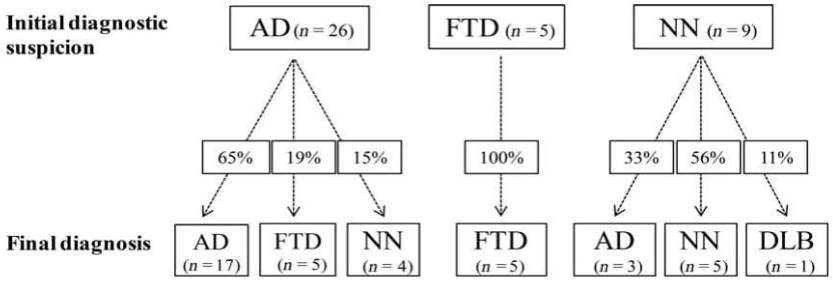

Figure 1 Diagnostic changes due to biomarker results. AD, Alzheimer's disease; FTD, frontotemporal dementia; NN, non-degenerative condition.

suspicion of FTD diagnosis (five bvFTD and one svPPA).

Change in therapeutic management

Biomarkers also contributed to treatment change in 32.5% of the participants (13 subjects) (Table 3). Cholinesterase inhibitors were initiated in four patients due to a change from an initial diagnosis of non-AD to a final diagnosis of AD/DLB. The treatment was not started in eight patients because biomarker results ruled out the initial diagnostic suspicion of AD. Cholinesterase inhibitor was withdrawn in one patient (with a diagnosis change from AD to a non-neurodegenerative disorder).

Diagnostic confidence before and after the biomarker results

The neurologist's diagnostic confidence was compared before and after biomarkers (Table 3). The initial diagnosis was confirmed after biomarkers in 27 patients. In the patients with AD, both CSF and amyloid-PET increased the diagnostic confidence (+8.8% and + 15%, respectively, $P < 0.01$). However, MTA assessment decreased it ($P < 0.01$) and FDG-PET showed no significant increase. When non-degenerative disorder was suspected, both CSF (+21.4%, $P < 0.05$) and amyloid-PET (+41.4%, $P < 0.01$) increased diagnostic confidence but no significant increase was found for MTA or FDG-PET. For suspected FTD, diagnostic confidence did not reach statistical significance although CSF and amyloid-PET showed an increase of up to 20%.

Patient tolerance of biomarkers

Patient tolerance showed no significant differences between biomarkers. However, FDG-PET (63%) and amyloid-PET (61%) showed the highest rate of non-discomfort and LP showed the highest percentage (26%) of mild discomfort (local pain during the LP or headache after it). The highest percentage of severe discomfort was for MRI (21%), mostly due to anxiety and claustrophobia (Table 4). A total of 79% of participants would surely repeat or probably repeat all tests with no significant differences between biomarkers (Table 4). A total of 8.1% of patients would not give consent to repeat the MRI.

Table 3 Diagnosis, treatment and diagnostic confidence changes before and after biomarker results

Pre-biomarker diagnosis	AD (n = 26)	FTD (n = 5)	NN (n = 9)
Diagnostic change	9 (34.6)	0 (0)	4 (44.4)
Treatment change	9 (34.6)	0 (0)	5 (55.6)
Pre-biomarker DC	77.4 ± 5	71 ± 31.9	53.6 ± 15.4
Post-CSF DC	86.2 ± 13.3	86 ± 8.2	75 ± 20
Change in DC	8.8 ± 13.8*	15 ± 37.4	21.4 ± 21**
Post-MTA score DC	57.6 ± 24.8	62 ± 24.1	65 ± 23.2
Change in DC	−19.7 ± 24.1*	−9 ± 49.4	11.4 ± 22.6
Post-FDG-PET DC	72.1 ± 19.5	82 ± 10.4	66 ± 31.5
Change in DC	−5.3 ± 20.7	11 ± 40.7	12.4 ± 28
Post-amyloid-PET DC	92.4 ± 6.6	87 ± 7.6	95 ± 8.7
Change in DC	15 ± 9.7*	16 ± 26.5	41.4 ± 15*

AD, Alzheimer's disease; CSF, cerebrospinal fluid; DC, diagnostic confidence; FDG, ^{18}F-fluorodeoxyglucose; FTD, frontotemporal dementia; MTA, medial temporal atrophy; NN, non-degenerative condition; PET, positron emission tomography; *$P < 0.01$; **$P < 0.05$. Data are given as n (%) and mean ± SD.

Discussion

From our results, the use of biomarkers increases the diagnostic confidence in patients with early-onset cognitive impairment. However, differences in diagnostic confidence were observed between biomarkers. CSF biomarkers and amyloid-PET increased it in AD, whereas MTA and FDG-PET did not demonstrate any added diagnostic confidence. In almost one-third of patients, clinical diagnosis and/or treatment changed after biomarker results. All tests were well tolerated.

Table 4 Distribution of tolerability and willingness to repeat biomarker procedures

Discomfort	LP	MRI	FDG-PET	Amyloid-PET
None	45.2	54.1	63.2	62.1
Mild	25.8	16.2	18.4	16.2
Moderate	16.1	8.1	13.2	13.5
Severe	12.9	21.6	5.2	8.2
Would repeat it?				
Yes, sure	80.6	70.3	81.6	81.6
Probably yes	9.7	16.2	10.5	10.5
Probably not	9.7	5.4	5.3	5.3
No, never	0	8.1	2.6	2.6

FDG, ^{18}F-fluorodeoxyglucose; LP, lumbar puncture; MRI, magnetic resonance imaging; PET, positron emission tomography. Data are given as %.

The percentage of diagnostic/therapeutic change due to biomarkers is in accordance with other publications although most of them evaluated it in late-onset AD [14–17]. These data together reflect the important impact of AD biomarkers in daily clinical practice in the diagnostic work-up of early-onset cognitive impairment.

Our data show that each biomarker has different added value to increase diagnostic confidence. Data on comparison of diagnostic confidence between all biomarkers are scarce. Some studies have been published on the impact of an isolated biomarker or two-biomarker comparison [8]. In our cohort, amyloid-PET and CSF increased the diagnostic confidence. The improvement in diagnostic confidence by amyloid-PET and its superiority over other biomarkers, such as FDG-PET, has also been suggested [15]. Previous studies also suggest that CSF biomarkers improve diagnostic confidence, with a high correlation with amyloid-PET results [13,14,18,19]. These data are in accordance with our results as both biomarkers reach good concordance and there are no differences in terms of added diagnostic value between them. In this context, there is no apparent benefit of using another biomarker if amyloid-PET or CSF biomarkers have been performed.

These biomarker differences on diagnostic confidence could be related to how informative they are about brain pathological changes in AD. CSF and amyloid-PET have better diagnostic confidence probably because they provide direct information related to the pathological brain changes that take place in AD, i.e. amyloid and tau deposition. In this respect, CSF gives more information than amyloid-PET because it provides data not only about amyloid but also about tau deposition, which should be considered as an added value with regard to other biomarkers. In addition, it is well known that the diagnostic accuracies of CSF and amyloid-PET are better than those of other biomarkers. Furthermore, they are in accordance with the *Consensus Report of the Working Group on Molecular and Biochemical Markers of AD* as definitely being considered diagnostic biomarkers. For both reasons, CSF and amyloid-PET could provide certainty to clinicians in determining or ruling out a diagnosis of AD [20–22].

In the present study, although some biomarkers increased diagnostic confidence up to 15%–20% in FTD, it did not reach statistical significance. We consider that a larger sample of patients with FTD would probably have allowed demonstration of the utility of these biomarkers in FTD diagnosis in line with previously published data [23].

Surprisingly, a reduction in diagnostic confidence after MTA assessment was observed, suggesting MTA to be less helpful in the early-onset cognitive impairment diagnosis framework. This is probably related to the lack of relevant MTA in our sample. This was expected as patients with early-onset AD have less hippocampal atrophy than those with late-onset AD and, when it is present, it overlaps with other neurodegenerative disorders, such as FTD. To the best of our knowledge, no studies of isolated MTA diagnostic confidence in early-onset AD have been performed, but Verhagen *et al.* determined that atrophy scales on MRI increased diagnostic confidence in cognitive impairment [17]. These data do not fit with ours probably due to several methodological differences, such as non-biomarker-supported diagnosis, inclusion of late-onset subjects and the use of various visual scales. For clinicians, CSF and amyloid-PET would be more reliable for the diagnostic process of early-onset cognitive impairment because they provide more certainty in establishing or ruling out AD.

Furthermore, in our cohort, the use of biomarkers led to a therapeutic change in one-third of the patients. These data are in accordance with previous studies that showed a similar proportion of treatment change when evaluating isolated amyloid-PET or CSF [15,16]. All of these data suggest that well-defined diagnosis in patients with early-onset cognitive impairment could have direct implications on their therapeutic management.

In terms of procedures, patient tolerance was good for all tests and thus, in general, no restrictions would be needed. In cases of known claustrophobia avoiding MRI should be considered.

The main limitation of our study is the small sample size. However, as far as we are aware, this is the first study cohort of patients with early-onset cognitive impairment in which all of the available diagnostic biomarkers have been performed and therefore

that compares diagnostic confidence of all biomarkers at the same time. Another limitation is the variability in diagnostic confidence between clinicians, which is related to the subjective condition of this variable itself.

In conclusion, these data suggest that amyloid-PET and AD core biomarkers in CSF are the more helpful and reliable biomarkers for AD diagnosis in early-onset cognitive impairment and that their use has direct implications for the clinical and therapeutic management of these patients. Further studies in larger and well-characterized cohorts are needed to determine the diagnostic utility and reliability of each biomarker in MCI and mild dementia with the aim of optimizing their diagnostic management.

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Disclosure of conflicts of interest

The authors declare no financial or other conflicts of interest.

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Treball número 2:

Hippocampal atrophy has limited usefulness as a diagnostic biomarker on the early onset Alzheimer's disease patients: A comparison between visual and quantitative assessment

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TITLE: Hippocampal atrophy has limited usefulness as a diagnostic biomarker on the early onset Alzheimer's disease patients: A comparison between visual and quantitative assessment

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ABSTRACT

NIA-AA diagnostic criteria include volumetric or visual rating measures of hippocampal atrophy (HA) as a diagnostic biomarker of Alzheimer's disease (AD). We aimed to determine its utility as a diagnostic biomarker for early onset Alzheimer's disease (EOAD) by assessing Medial Temporal Atrophy (MTA) and hippocampal volume (HV) determination. MTA score and HV quantified by FreeSurfer were assessed in 140 (aged ≤65) subjects with biomarker supported diagnosis: 38 amnesic (A-EOAD), 20 non-amnesic (NA-EOAD), 30 late onset AD (LOAD), 20 fronto-temporal dementia (FTD) and 32 healthy controls (HC). The results showed that the proportion of MTA≥1.5 was higher on LOAD and FTD than EOAD and HC but none of the MTA thresholds (≥1, ≥1.5 and ≥2) showed acceptable diagnostic accuracy. LOAD had lower HV than the other groups. A-EOAD HV was lower than NA-EOAD and HC but equal to FTD. The 6258mm3 cut-off showed good diagnostic accuracy between A-EOAD and HC. Both tools showed a moderate inverse correlation. In conclusion, MTA has a limited diagnostic utility as an EOAD biomarker as it does not discriminate AD from FTD or HC in initial symptomatic stages. HV may discriminate A-EOAD from HC but not from FTD.

Key Words: Alzheimer's disease, Frontotemporal dementia, Atrophy, Magnetic Resonance Imaging

Abbreviations: AD, Alzheimer's disease; EOAD, Early Onset Alzheimer's disease; A-EOAD, Amnesic Early Onset Alzheimer's disease; NA- EOAD, Non-Amnesic Early Onset Alzheimer's disease; LOAD, Late-onset Alzheimer's disease; FTD, Fronto-temporal dementia; bvFTD, behavioral variant of Fronto-temporal dementia; nfvPPA, non-fluent variant of Primary Progressive Aphasia; svPPA, semantic variant of Primary Progressive Aphasia; HC, healthy controls; HA, Hippocampal atrophy; HV, Hippocampal volume; MTA, Medial temporal atrophy; MMSE, Mini Mental State Examination; FAQ, Pfeiffer Functional Activities Questionnaire; MCI, mild cognitive impairment.

1.INTRODUCTION

Up to 10% of subjects with neurodegenerative dementias have an early onset disease, defined as a clinical onset below 65 years (Garre-Olmo J et al., 2010). The most frequent cause of early-onset dementia is Alzheimer´s disease (AD), usually presenting with progressive anterograde episodic memory impairment. However, non-amnestic clinical presentations, are also commonly seen in early onset AD (EOAD) and frequently overlap with other neurodegenerative dementias such as frontotemporal dementia (FTD) leading to greater rates of misdiagnosis and diagnostic delay (Balasa M et al., 2011; Mendez MF et al., 2008). In this context the use of specific biomarkers is crucial in achieving an accurate diagnosis.

Hippocampal atrophy (HA) is a well-recognized feature of AD considered a neurodegeneration biomarker in current NIA-AA diagnostic criteria (Apostolova LG et al., 2006; Sarazin M et al., 2010; Albert MS et al., 2011; McKhann GM et al., 2011). Medial Temporal Atrophy (MTA) visual rating scale is a popular HA evaluation instrument, although its utility in EOAD is still unclear (Scheltens Ph et al., 1992). Since quantitative volumetric analysis is time consuming, semiautomatic methods have been developed. Data regarding its reliability in early symptomatic stages is lacking (Cuingnet R et al., 2011).

In this study we sought to elucidate the differences in HA between LOAD, EOAD and FTD, and compare visual and semiautomatic quantitative assessment diagnostic accuracy. We hypothesized that quantitative analysis would be more useful, especially in amnesic EOAD.

2. METHODS

2.1 Subjects:

The study was approved by the Hospital Clinic Barcelona Ethics Committee and all participants gave written informed consent. One hundred forty subjects evaluated at the Alzheimer's disease and other cognitive disorders Unit at Hospital Clínic de Barcelona were enrolled on this retrospective cross-sectional study. All subjects underwent a complete neurological and neuropsychological evaluation, 3T brain MRI and a spinal tap for the determination of AD CSF biomarkers. All subjects scored ≥20 on the Mini Mental

State Examination (MMSE), had a clinical onset before 65. Patients were classified into 3 groups:

1) EOAD group (n=58): 28 of them with mild cognitive impairment (MCI) (Pfeiffer Functional Activities Questionnaire (FAQ)≤6) and 30 with mild dementia (Pfeffer RI et al., 1982). All subjects had a typical AD CSF biomarker profile. So, all EOAD fulfilling the NIA-AA diagnostic criteria for MCI due to AD or AD dementia (Albert MS et al., 2011; McKhann GM et al., 2011). Based on their clinical presentation, EOAD participants were further classified into two subgroups: amnesic (A-EOAD, 38 subjects) and non-amnesic variant (NA-EOAD, 20 subjects).
2) LOAD group (n=30): All subjects had a typical AD CSF biomarker profile. Nineteen of them fulfilled the NIA-AA diagnostic criteria for MCI due to AD and eleven for AD dementia (Albert MS et al., 2011; McKhann GM et al., 2011).
3) FTD group (n=20): Six behavioral variant of FTD (bvFTD), seven non-fluent variant for primary progressive aphasia (nfvPPA) and seven semantic variant of primary progressive aphasia (svPPA) (Rascovsky K et al., 2011; Gorno-Tempini ML et al., 2011). A *C9orf72* expansion was present in two cases of bvFTD and three nfvPPA subjects had *GRN* mutation. All FTD subjects had normal AD CSF biomarkers levels.
4) Healthy controls (n=32): with no cognitive complaints, cognitive performance within normative range and normal AD CSF biomarkers. They were classified as young HC (aged under 65) (n=16) and older ones (aged over 65) (n=16)

2.2 Genetic and CSF biomarkers determination

APOE genotype was determined through the analysis of rs429358 and rs7412 by Sanger sequencing.

All subjects underwent a spinal tap. Levels of amyloid β ($A\beta_{42}$), total-tau (T-Tau), and phosphorilated-tau (P-Tau) were measured using commercial sandwich ELISA kits (Fujirebio, Gent, Belgium). The CSF cut-off values determined by our laboratory were used to classify subjects according to NIA-AA criteria (Balasa M et al., 2014).

2.3 Brain MRI imaging

For each subject, high-resolution sagital T1-weighted MRI images were acquired in a 3Tesla scan (Siemens Magnetom Trio, Erlangen, Germany) at the Magnetic Resonance Image Core Facility, using proprietary three-dimensional magnetization-prepared rapid-acquisition gradient echo: MPRAGE sequences (TR=2300 ms; TE=2,98 ms; acquisition matrix 256 x 256, voxel size 1x 1 x 1).

2.4 Visual assessment

We used T1-coronal images on midbrain to score right and left hippocampus; we scrolled through the whole hippocampus and then selected the main assessment slice in the middle of the hippocampal body, in front of the pons. It was rated according to the five-point scale developed and validated by Scheltens *et al*. and then averaged to obtain a single MTA value for each subject (Scheltens Ph et al., 1992, Harper et al. 2016). An expert radiologist specialized in dementia neuroimaging (N.B.) and a trained neurologist (N.F.) blinded to diagnosis and clinical data, visually assessed MRI images.

2.5 Quantitative assessment

T1 sequences were analyzed with FreeSurfer v5.3 in neuGRID platform to obtain the HV with the pipeline nG+FreeSurfer+5.3.0+Diagnostic+v05.xml. This pipeline relies on Freesurfer-ReconAll 5.3.0 in cross sectional mode (Fischl B et al., 2004; Reuter M et al., 2012; Cover KS et al., 2016). Freesurfer segments the hippocampi of 3D T1-weighted structural brain MRI scans. The pipeline involves intensity non-uniformity correction, affine transformation to MNI template, intensity normalization, skull stripping, removal of non-brain tissue, linear and non-linear transformations to a probabilistic brain atlas and labelling of subcortical structures. The right label per each single voxel is determined using spatial localization priors (Fischl B et al., 2002). The hippocampal volumetric values in mm^3 are contrasted against a normative population of 238 healthy controls of ADNI. All automated hippocampal segmentations were performed on 64-bit Linux machines using the neuGRID web-portal (www.neugrid4you.eu). NeuGRID allows to efficiently analyze large amount of imaging data with more than 5000 CPU cores and 20TB of physical space (Redolfi A et al., 2013; Redolfi A et al., 2015). The neuGRID

platform provided a final report directly to the physician reporting the volumetric information, the percentile assessments, as well as pictures of the segmented hippocampi allowing a direct evaluation of the FreeSurfer segmentation results (see S*upplementary material Fig.S1*).). Additionally, we also analyzed HV using FreeSurfer's standard approach, i.e., native-space volume normalized by total intracranial volume.

2.6 Statistical Analysis

Statistical analysis was conducted using Stata/IC 14.2. Categorical data was analyzed by χ^2 test and quantitative data by ANOVA and Student's *t*-test with Bonferroni *post-hoc* procedure. *APOE* Ɛ4 status was dichotomized as carrier/non-carrier and variables were compared in ε4 carrier and non-carrier groups using ANOVA. Inter-rater reliability of left, right and averaged MTA score was determined by Kappa index and intraclass correlation coefficient (ICC) by a two-way random, absolute for single-measures [ICC (2,1)] and average measures ICCs [ICC(2,k)]. The quantitative assessment's accuracy was estimated using receiver operator characteristics (ROC). Best thresholds were selected maximizing sensitivity and specificity. Pearson correlation coefficients evaluated the association between visual and quantitative assessments.

3. RESULTS

3.1 Demographics

LOAD and old HC were older than other groups as expected. Groups showed no differences in gender *(Table 1)*. AD and FTD groups showed lower MMSE scores (p<0.01) compared to HC. The presence of *APOE* Ɛ4 allele was highest among A-EOAD (65.8%) than in FTD and HC groups (p<0.05), including significant differences between A-EOAD and NA-EOAD (p=0.038).

3.2 Visual assessment

3.2.1 Descriptive data

MTA scores showed substantial agreement between both raters: 88% of total agreement (k=0.83) on the left, 88% (k=0.84) on the right and 83% (k=0.78) on the averaged MTA). Both single and average ICC values were >0.9 which is considered an excellent

correlation between both raters (see *Supplementary material Table S1*). Most discrepancies were found on 0 and 1 scoring and were solved by consensus.

No significant differences were observed between right and left Scheltens' score, neither between the whole groups FTD (nfvPPA, svPPA and bvFTD) on MTA. The comparison of MTA scores between MCI due to AD and mild AD dementia subjects did not show differences, neither considering *APOE* Ɛ4 status.

The distribution of the visual MTA assessment is displayed in *Figures 1* and *2.* Proportion of participants with MTA score ≥1.5 was higher in LOAD (77%, $p<0.01$) and FTD (70%, $p<0.01$) than A-EOAD (34.3%), NA-EOAD (30%) and HC groups (6,3%). FTD was the only group with MTA≥3.5 (15%). A-EOAD showed differences compared to HC ($p=0.032$) but not to NA-EOAD or FTD.

3.2.2 Diagnostic performance

We evaluated diagnostic sensitivity and specificity for different thresholds of the MTA score among AD and FTD groups (*Table 2a*). MTA performed better at discriminating LOAD and FTD from HC than EOAD from HC. However, no threshold reached 80% performance on both sensitivity and specificity. In addition, MTA did not reach 60% diagnostic accuracy at discriminating between AD groups from FTD (*Table 2b*).

3.3 Quantitative assessment

3.3.1 Descriptive data

There were no differences in right and left HV. Thus, averaged HV were used for subsequent analyses. No significant differences were found on mean HV values among FTD clinical variants (nfvPPA, svPPA and bvFTD) neither between MCI due to AD and mild dementia due to AD. HV considering *APOE* Ɛ4 status did not show differences between groups.

The distribution of the HV by different diagnostic groups is shown in *Figure2*. We observed significant differences on the average HV between groups ($p<0.001$). The *post hoc* analysis showed that HV LOAD (4890±866 mm^3) was smaller than the other groups

(p<0.05). A-EOAD HV (5568±663 mm^3) was smaller than NA-EOAD (6260±872mm^3) (p=0.019) and HC (6766± 711mm^3) (p<0.001), but we did not find differences between A-EOAD and FTD groups (5671.1mm^3±1109 mm^3). No differences between NA-EOAD and HC or FTD were found. FTD had smaller HV than HC (p=0.001). Furthermore, the distribution and means of the HV by different diagnostic groups using total intracranial volume normalization is shown as S*upplementary material Table S2 and Fig.S2.*

3.3.2 Diagnostic performance

ROC curves were used to determine HV threshold with a better diagnostic performance. The 5838 mm^3 threshold distinguished between LOAD and HC with very good sensitivity (100%) and specificity (87%). HV threshold of 6259 mm^3 showed good sensitivity (88%) and specificity (87%) to distinguish A-EOAD from HC (*Figure3.1a*). For identifying NA-EOAD *versus* HC, the best threshold was 6745 mm^3, although it showed suboptimal sensitivity (69%) and specificity (70%) (*Figure3.1b*). The best HV cut-off for FTD versus HC was 6365.5 mm^3 (sensitivity 82.3% and specificity 75%) (*Figure3.1c*). We also compared AD groups to FTD (*Figure 3.2a and 3.2b*). The best cut-off for comparing A-EOAD and FTD was 5763.5 mm^3 and for comparing NA-EOAD *versus* FTD was 5845 mm^3, although both showed sensitivity and specificity below 70%. The 5124 mm^3 threshold distinguished LOAD from FTD with 75% sensitivity and 63% specificity. Moreover, diagnostic performance of HV using total intracranial volume normalization is shown in *Supplementary Material Fig.S3.*

3.4 Correlation between quantitative and visual assessment

There was a significant moderate inverse correlation (r=-0.537, p<0.001) between MTA score and the quantitative HV evaluation.

4. DISCUSSION

In this study we compared a visual scale and a semiautomatic tool for measuring HA in patients with EOAD, LOAD and FTD in order to determine the diagnostic accuracy of both tools in early symptomatic stages. In our cohort, MTA scale had little utility as a

diagnostic biomarker and the quantitative measurement of HV was useful only in distinguishing LOAD and A-EOAD from HC.
To our knowledge this is the first study to compare both visual and volumetric assessment in a well-characterized biomarker-confirmed cohort of LOAD, EOAD and FTD patients.

Since its development, Scheltens' HA visual rating scale has been validated in numerous studies as a good predictor of progression from MCI to AD dementia and to discriminate AD from HC and has also been proved to correlate with volumetric methods (Heo JH et al., 2013; Wahlund LO et al., 2000). The MTA score has been proposed as the best marker of HA and the MTA≥1.5 cut-off has been recommended to be used for AD diagnosis under the age of 75 (Van de Pol LA et al., 2014). In the case of volumetric assessment, it is not clear yet which HV cut off should be used.

In our study, MTA was not able to accurately discriminate HC from AD or FTD patients, since specificity and sensitivity did not reach the 80% threshold for any of the groups (Consensus report of the Working Group on: molecular and biochemical markers of Alzheimer's disease, 1999). The quantitative assessment only achieved a good diagnostic performance in discriminating LOAD and A-EOAD from HC with little discriminative capacity between the other comparisons.

Most previous studies point to the fact that both visual scales and quantitative analysis are able to discriminate between AD and HC, despite relevant variability in terms of diagnostic performance and they mostly focus on LOAD patients (Heo JH et al., 2014; Cavedo E et al., 2014). In reference to LOAD, our results are in line with previous publications in terms of sensitivity albeit greater specificity in our cohort. (Harper et al., 2016) This could be explained by the characteristics of our older HC group in whom preclinical AD had been carefully ruled out through the use of CSF biomarkers and an extensive neuropsychological evaluation. In both visual scales and quantitative analysis, the diagnostic accuracy is better in LOAD than EOAD, as well as it is better in A-EOAD than in NA-EOAD. It makes sense, since LOAD patients mostly present amnesic clinical phenotype and have more HA whereas EOAD patients frequently have a non-amnestic

clinical profile related to less HA (Balasa M et al., 2011; Koedam EL et al., 2010; Phillips J, 2018). Poor MTA diagnostic accuracy results on EOAD may be due to the fact we have only included patients in early symptomatic stages (i.e, MCI and mild dementia, MMSE≥20). Is well known that patients in advanced stages of the disease have more HA, thus, in consequence, assessing MTA at these stages could increase its discriminative power; but on the daily clinical practice it is in the early stages of the disease when the differential diagnosis is more complex and it is precisely where biomarkers should be more useful and make the difference. On the other hand, most studies using Scheltens' MTA scale do not have CSF results and the AD diagnosis is based only on clinical criteria (Cuingnet R et al., 2011; Heo JH et al., 2013; Wahlund LO et al., 2000; Van de Pol LA et al., 2014; Duara R et al., 2013; Ferreira D et al., 2015; Varon D et al., 2015; Pereira JB et al., 2014; Shen Q et al., 2011; Ridha BH et al., 2007). We consider this fact to be a significant caveat since trying to establish AD diagnosis without biological confirmation of the disease can lead to higher rates of misdiagnosis (Beach TG et al., 2010). Claus *et al* reported good diagnostic accuracy (83.3% sensitivity and 86.4% specificity) for MTA≥1 at discriminating 18 EOAD patients from subjective cognitive impairment subjects (Claus JJ et al.,). Their data does not fit with our results, which could be explained by several methodological differences such as: the use of computerized tomography (CT) scan, the lack of biomarker supported diagnosis, the wide range of clinical severity stages included in the sample and its small size.

Volumetric assessment is expected to provide an added value in AD diagnosis (Bosco P et al., 2017). Several techniques have been developed to achieve the most accurate measurement and many comparisons of both tools have been published (Cuingnet R et al., 2011; Heo JH et al., 2013; Wahlund LO et al., 2000; Van de Pol LA et al., 2014; Duara R et al., 2013; Ferreira D et al., 2015; Varon D et al., 2015; Pereira JB et al., 2014; Shen Q et al., 2011). However, comparing them is difficult because of the heterogeneity of study samples and imaging techniques used. Cuingnet *et al*. compared the diagnostic performance of different quantitative methods, including volumetric measurement of HV of clinically diagnosed MCI and mild AD patients aged 55-90 (Cuingnet R et al., 2011). HV evaluation was as sensitive as other methods but however less specific: 63% sensitivity and 80% specificity on distinguishing HC from AD patients and 73%

sensitivity and 74% specificity between HC and MCI. By contrast, our data show acceptable diagnostic performance of the volumetric analysis to distinguish A-EOAD from HC. That difference can be explained also by methodological differences such as no biomarker supported diagnosis.

When comparing both LOAD and EOAD with FTD patients, visual and quantitative HA assessment does not appear to be a good diagnostic biomarker. This data fits well with previous works that show similar HA on both disorders (Van de Pol LA et al., 2006; Hornberger M et al., 2012). The relevant MTA and its wide distribution found on FTD could be related to the heterogeneity and the fast disease progression of the FTD itself.

The clinical overlap between NA-EOAD and FTD sometimes leads to differential diagnosis difficulties in clinical practice and highlights the crucial importance of using disease-specific biomarkers (Beach TG et al., 2012). Unfortunately, in our cohort, neither visual nor quantitative assessment performed optimally at differentiating them.

In line with previous studies mostly performed in late-onset cognitive impairment, we observed a moderate correlation between MTA visual rating and volumetric techniques (Dhikav V et al., 2017). The moderate correlation could be explained because MTA score is based mostly on a single slice while HV is a three-dimensional measure. These data suggest that both techniques may perform in a different way with regard to HV measurements, therefore caution must be taken before considering them equivalent.

Furthermore, variability through different HV quantification methods has been previously described (Buckner et al. 2004). Conversely, both NeuGRID platform and total intracranial volume normalization methods provided quite similar results. It suggests that HV diagnostic performance is consistent across analysis methods.

Our results would support the idea that visual MTA rating may play a limited role in the clinical diagnosis of EOAD, in particular in early clinical stages. With regards to volumetric assessment, it would only show an advantage compared to visual rating in discrimination between LOAD and A-EOAD from HC. Current criteria for the clinical diagnosis of AD includes HA as a neurodegeneration biomarker (Albert MS et al., 2011; McKhann GM et al., 2011). In light of our results showing low discriminative capacity of

HA between groups, this feature should be interpreted with caution in early-onset cognitive impairment especially in patients with non-amnestic presentations.

The main limitation of our study is the relatively small sample size, although, as far as we are aware this is the larger cohort study focused on EOAD patients reported until now. On the other hand, we have only used one semiautomatic volumetric analysis and it is possible that other volumetric techniques for evaluating HA may show different diagnostic performances.

4.1 Conclusion

In conclusion, the utility of HA as a diagnostic biomarker in our cohort of EOAD patients, is limited. Further studies in larger and well-characterized cohorts are needed to determine the diagnostic utility of HA as a biomarker in the early stages for EOAD patients.

Disclosure statement:
Authors state that there are no conflicts of interest to disclose.

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Table 1.

	A-EOAD (n=38)	**NA-EOAD** (n=20)	**FTD** (n=20)	**Young HC** (n=16)	**LOAD** (n=30)	**Older HC** (n=16)
Gender (% Female)	57.9	60	45	62.5	60	46.7
Age Mean	61.3±5	59.7±5.7	61.1±4.4	57.5±3.3	71.3±5.2[a]	74.7±3.9[a]
AAO Mean	58.3±4.7	57.2±5.5	58.1±4.4	-	72.2±4.9[b]	-
Time to diagnosis	3±1.5	2.5±1.3	3±2.2	-	2.4±2.2	-
MMSE score	24.3±2.6	25.1±3	25.8±2.8	29±1.2[c]	24.2±3	28.4±0.7[c]
***APOE* Ɛ4** (% positive)	65.8[d]	35	20	18.8	55.2[f]	6.7
CSF Aβ42 (pg/mL)	397.6±108[e]	366.28±124[e]	851.1±305	893.2±247	358.2±105[e]	733±185
CSF P-Tau (pg/mL)	108.6±36[e]	112.1±44[e]	48.6±18	51.4±9	95±47[e]	59.3±17
CSF T-Tau (pg/mL)	748.3±426[e]	701.9±382[e]	327.6±158	224.3±52	677±439[e]	299.1±116

Data are presented as means±standard deviation. A-EOAD, Amnesic Early Onset Alzheimer Disease; NA-AEOD, Non-Amnesic Early Onset Alzheimer Disease; FTD, Frontotemporal Dementia; HC, Healthy Controls; AAO, Age At Onset.

[a] Statistically significant (p<0.01) differences compared to A-EOAD, NA-EOAD, FTD, Young HC

[b]Statistically significant (p<0.01) differences compared to A-EOAD, NA-EOAD, FTD.

[c] Statistically significant (p<0.01) differences compared to A-EOAD, NA-EOAD, FTD and LOAD.

[d]Statistically significant (p<0.05) differences compared to NA-EOAD, FTD, LOAD and young and older HC.

[e]Statistically significant (p<0.05) differences compared to FTD, young and older HC.

[f]Statistically significant (p<0.05) differences compared to older HC.

Table 2.

2a) Diagnostic accuracy for MTA visual rating of diagnostic groups vs HC.

	A-EOAD (n=38)	NA-EOAD (n=20)	LOAD (n=30)	FTD (n=20)
MTA≥1	Se 58% Sp 69%	Se 45% Sp 69%	Se 90% Sp 56%	Se 80% Sp 69%
MTA≥1,5	Se 34% Sp 93%	Se 30% Sp 93%	Se 77% Sp 94%	Se 70% Sp 94%
MTA≥2	Se 29% Sp 94%	Se 15% Sp 94 %	Se 63% Sp 100%	Se 50% Sp 94%
AUC	0.67 (0.54-0.81)	0.63 (0.46-0.80)	0.88 (0.79-0.98)	0.85 (0.72-0.97)

2b) Diagnostic accuracy for MTA visual rating of AD group vs FTD group.

	A-EOAD (n=38)	NA-EOAD (n=20)	LOAD (n=30)
MTA ≥1	Se 58% Sp 20%	Se 45% Sp 20%	Se 80% Sp 10%
MTA≥1,5	Se 34% Sp 30%	Se 30% Sp 30%	Se 70% Sp 23%
MTA ≥2	Se 29% Sp 50%	Se 15% Sp 50%	Se 50% Sp 37%
AUC	0.28 (0.14-0.43)	0.24 (0.09-0.31)	0.53 (0.35-0.68)

A-EOAD, Amnesic Early Onset Alzheimer's Disease; NA-EOAD, Non-Amnesic Early Onset Alzheimer's Disease; FTD, Frontotemporal Dementia; HC=Healthy Controls; MTA, Medial temporal Atrophy score; Se, Sensitivity; Sp, Specificity.

Figure 1. Distribution of MTA scoring for diagnostic groups and HC (percentage, %).

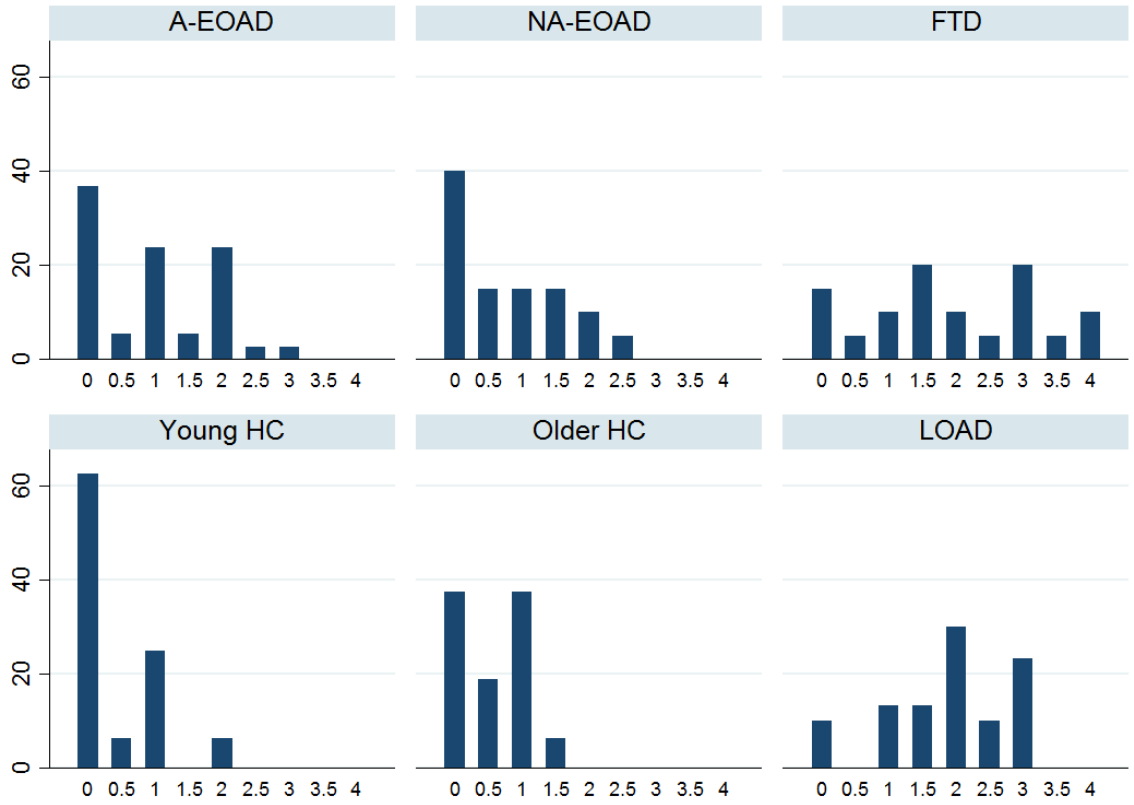


Figure 2. Box plots of the MTA and HV distribution depending on the diagnosis.

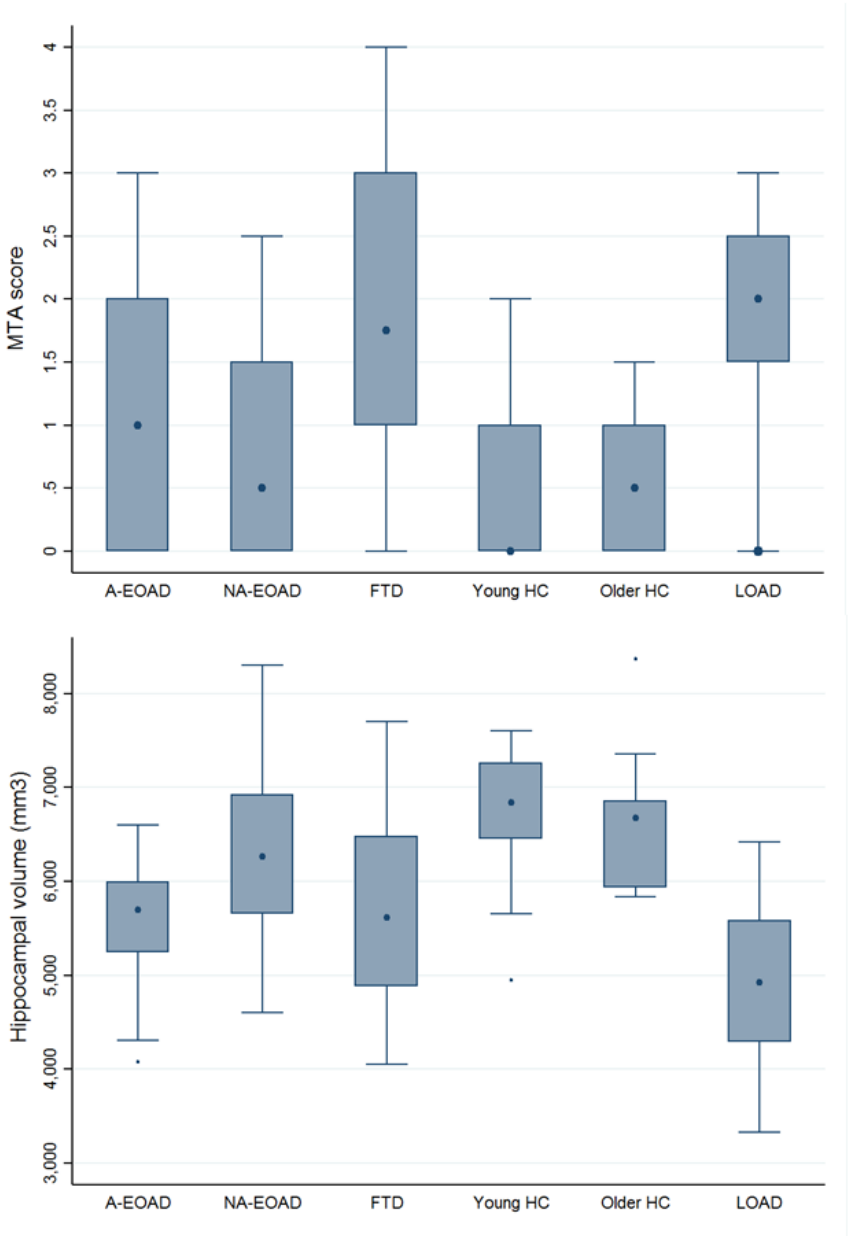
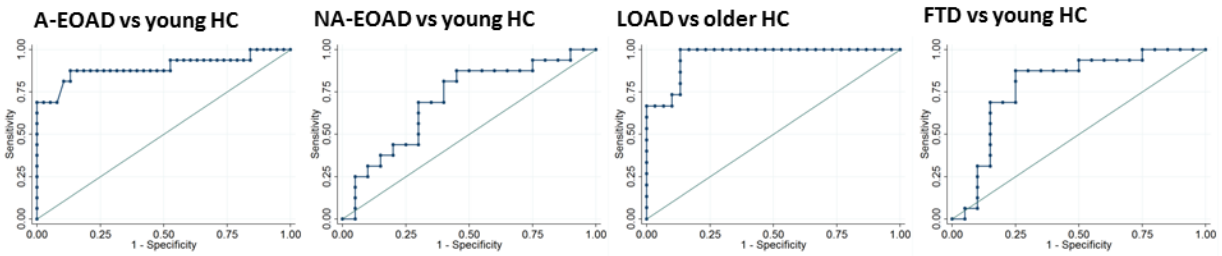


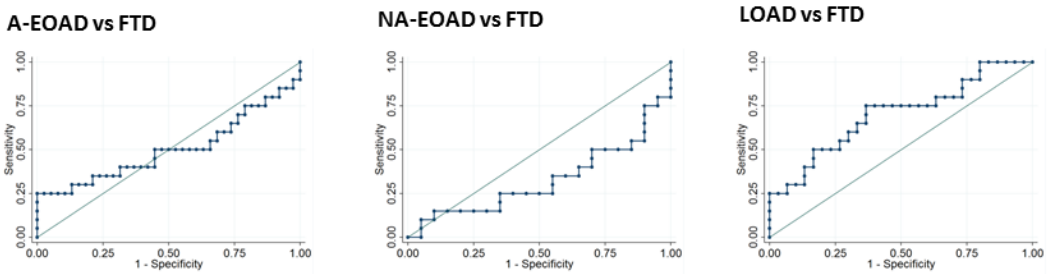
Figure 3. Diagnostic performance of HV quantitative assessment

3a) ROC curves of HV of diagnostic groups compared to HC



[AUC: 0.90 (0.78-1) *p*<0.01] [AUC: 0.70 (0.53-0.88) p<0.05] [AUC: 0.97 (0.91-1) *p*<0.01] [AUC: 0.791 (0.63-0.95) *p*<0.01]

3b) ROC curves of HV of AD groups compared to FTD



[AUC: 0.50 (0.33-0.68) *p*=0.97] [AUC: 0,328 (0,156-0,499) *p*=0,06] [AUC: 0.33 (0.16-0.5) *p*=0.06]

Supplementary Material Table S1 Inter-rater reliability measures: total agreement, Kappa index and intraclass correlation coefficient.

Scale	**Single mesures ICC** 2 rater *n*=141	**Average-Mesures ICC** 2 rater *n*=141
Left MTA	0.94 (0.92-0.95)	0.97 (0.96-0.98)
Right MTA	0.91 (0.88- 0.94)	0.96 (0.88-0.94)
Averaged MTA	0.94 (0.92-0.96)	0.97 (0.96-0.98)

Scale	**Agreement** (%) 2 rater *n*=141	**Kappa** 2 rater *n*=141
Left MTA	88	0.83
Right MTA	88	0.84
Averaged MTA	83	0.78

Supplementary Material Table S2. Mean hippocampal volumes by groups using total intracranial volume normalization.

	HV mean (mm^3) ±SD
A-EOAD (n=38)	3171±365.7
NA-EOAD (n=20)	3506±434
LOAD (n=30)	2860±377
FTD (n=20)	3241±544
Young HC (n=16)	3809±376
Older HC (n=16)	3646± 418

Data are presented as means±standard deviation; A-EOAD, Amnesic Early Onset Alzheimer Disease; NA-AEOD, Non-Amnesic Early Onset Alzheimer Disease; FTD, Frontotemporal Dementia; HC, Healthy Controls; SD, standard deviation

Supplementary Material Figure S1. Mean hippocampal volumes by groups using total intracranial volume normalization.

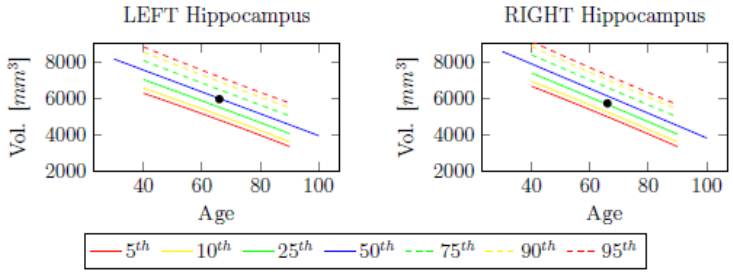
Freesurfer Report

Subject info

Patient ID: 20091023_113432MPRAGESAGIPATISOPROVAs002a1001 **Sex:** M **Age:** 66

Freesurfer Recon-All[1]

Model: Recon-All FS v5.3
Left Volume: 5947 mm^3 **Right Volume:** 5714 mm^3



Every colored line represents the *Prediction Percentile* for the hippocampal volume of a normative dataset made of 238 *ADNI* healthy elderly controls. In these graphs the left and right hippocampal volumes of the patient are represented by black dots.

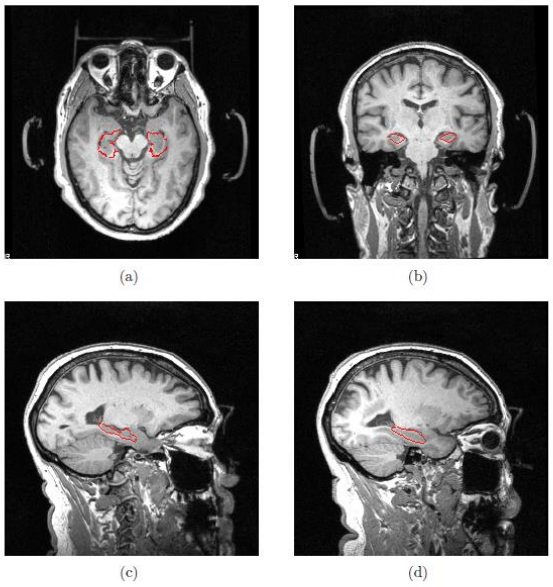
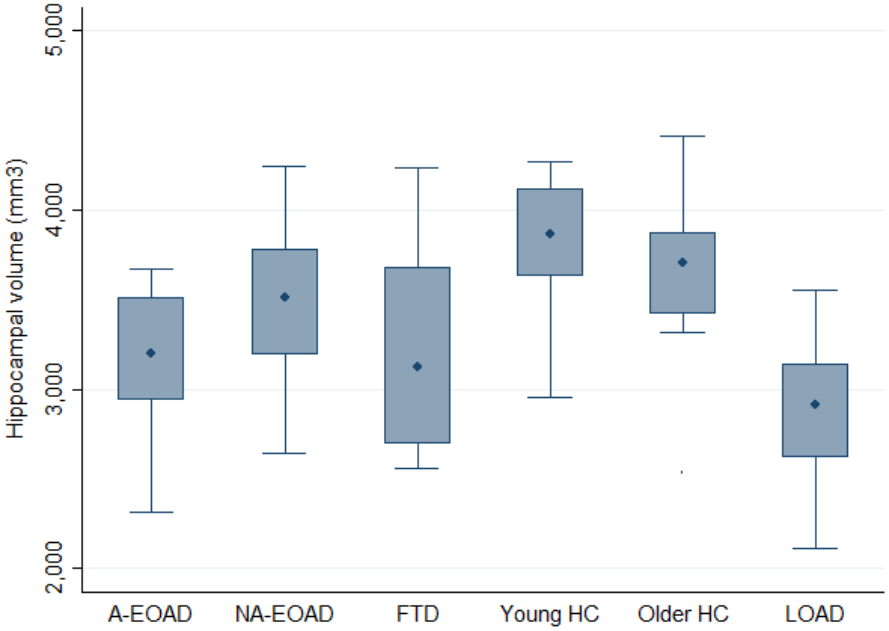
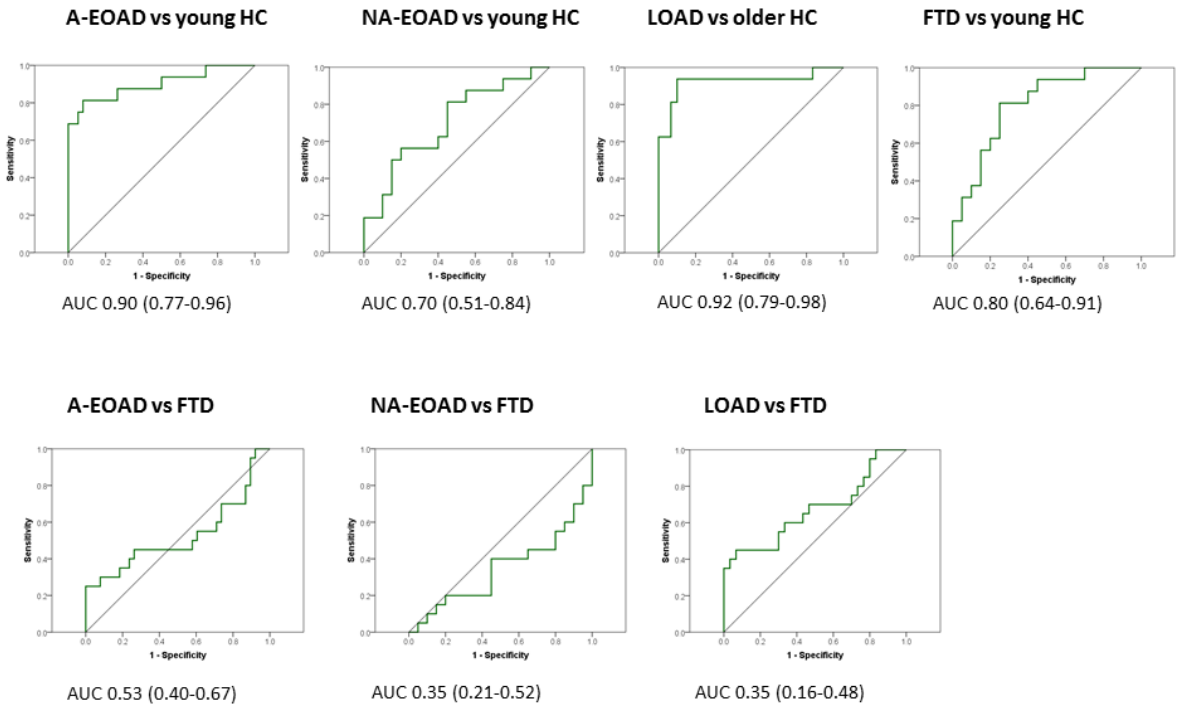


Figure 1: Segmentation of the brain scan in the native space. The letter "R" identifies the Right direction. (a) Axial view. (b) Coronal view. (c) Sagittal view centered on the Right Hippocampus. (d) Sagittal view centered on the Left Hippocampus.

Supplementary Material Figure S2. Distribution of hippocampal atrophy by groups using total intracranial volume normalization



Supplementary Material Figure S3. ROC curves of hippocampal volume of diagnostic groups using total intracranial volume normalization



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Treball número 3:

Diagnostic accuracy of MRI visual rating scales in the diagnosis of early onset cognitive impairment.

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-Under Review-

Title: Diagnostic accuracy of MRI visual rating scales in the diagnosis of early onset cognitive impairment.

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Abstract

The diagnosis of incipient symptomatic stages of early-onset dementia is challenging. The magnetic resonance imaging (MRI) is an easy-access biomarker. We evaluated the visual atrophy scales usefulness in two hundred subjects: eighty-four early onset Alzheimer disease (AD) patients (48 amnestic, 22 non-amnestic, 14 autosomal-dominant), 25 frontotemporal dementia (eleven behavioral variant (bvFTD), nine semantic variant of primary progressive aphasia (svPPA), five non-fluent aphasia (nfvPPA), seven mutation carriers), 25 mild cognitive impairment due to non-degenerative disorders and 59 controls. All had MMSE≥18, 3T-brain MRI and biomarker-supported diagnosis. Two raters evaluated six frontal, temporal and parietal scales. Inter-rater reliability and diagnostic performance in terms of area under the receiver-operator curves and balanced accuracy were analyzed. Best scales to discriminate AD from controls were the anterior cingulate scale for amnestic and the posterior one for non-amnestic. Anterior temporal scale was the best for bvFTD and svPPA, anterior cingulate scale for nfvPPA and all scales for the genetic ones. However, no scale demonstrated good performance at discriminating AD from FTD or non-degenerative disorders. The clinicians should interpret with caution atrophy scale assessment in subjects with early-onset cognitive impairment given that none of the evaluated scales met the requirements for being a diagnostic biomarker.

Key Words: Alzheimer's disease, Frontotemporal dementia, Atrophy, Magnetic Resonance Imaging

Abbreviation list:

AC = anterior cingulate; AD = Alzheimer's disease; A-EOAD = amnesic early onset AD; ADAD = Autosomal dominant Alzheimer's disease; AUC = area under the receiver-operator characteristic curve; AT = anterior-temporal scale; CSF = cerebrospinal fluid; EOD = early onset dementia; FI = fronto-insular scale; FTD = frontotemporal dementia; bvFTD = behavioral variant of FTD; svPPA = semantic variant of primary progressive aphasia; nfvPPA = non-fluent variant of primary progressive aphasia; ICC = intraclass correlation coefficient; ICC(2,1) = two-way single measures ICC; ICC(2,k)= two-way average measures ICC based on k raters; MCI = mild cognitive impairment; MMSE = Mini Mental State Examination; MTA = medial temporal lobe atrophy, NA-EOAD = non-amnesic early onset AD; OF = orbito-frontal; PA = posterior atrophy; amyloid-PET = amyloid tracer-positron emission tomography

Introduction

Early onset dementia (EOD) can reach up to 10% of dementia cases (Garre-Olmo *et al*., 2010). The clinical diagnosis of EOD is frequently challenging and might lead to relevant delay until an accurate diagnosis (Mendez *et al*., 2014).

Different imaging, genetic and biochemical biomarkers have been developed and included in the Alzheimer's disease (AD) and frontotemporal dementia (FTD) current diagnostic criteria (Albert *et al*., 2011; McKhann *et al*., 2011; Rascovsky *et al*., 2011; Gorno-Tempini *et al*., 2011). Structural MRI is the most available biomarker. Although volumetric quantification methods have been developed, they are complex and time-consuming making them challenging to integrate into clinical practice. For that, the visual assessment is the more used tool for brain atrophy evaluation in clinical environments.

Visual assessment has been demonstrated to be useful in late-onset patients (Duara *et al*., 2013; Ferreira *et al*., 2015; Ten Kate *et al*., 2017; Claus *et al*., 2017). Instead, its usefulness on EOD has not been widely assessed. In a recent study, an extensive and well-structured rating protocol was applied to a mostly focused EOD cohort, but it included wide range of disease stages (Harper *et al*., 2016). In this context, they concluded that Fronto-Insular (FI) and Medial Temporal Atrophy (MTA) were the best scales to differentiate AD and FTD from controls.

However, the moment of the first evaluation is when these scales can be most needed to be of help, usually in initial symptomatic stages, mild cognitive impairment (MCI) and mild dementia. In this sense, we aim to elucidate the diagnostic performance of visual rating assessment on MRI in initial stages of the most frequent neurodegenerative EOD (AD and FTD) with a wide range of clinical phenotypes, in both sporadic and genetically determined cases.

Materials and methods

Subjects:

Two hundred subjects evaluated at the Alzheimer's disease and other cognitive disorders Unit at Hospital Clínic de Barcelona were enrolled in this cross-sectional study. The study was approved by the Hospital Clínic Barcelona Ethics Committee and

all participants gave written informed consent. All subjects had a clinical onset before 65 years and Mini Mental State Examination (MMSE)≥18 and were selected from the Early-onset Dementia Cohort and the Genetic counselling program for familial dementias (PICOGEN) (Fortea *et al*., 2011). All of them had a neurological and neuropsychological evaluation, 3T brain MRI and biomarker-supported diagnosis.

Patients were classified into the following groups:

1) Sporadic early onset AD (EOAD) (n=70): Twenty-seven with MCI (Pfeiffer Functional Activities Questionnaire FAQ≤6) and 43 with mild dementia (FAQ>6) (Pfeffer *et al*., 1982). All subjects had typical AD core cerebrospinal-fluid (CSF) biomarkers profile (n=65) or positive amyloid-PET (amyloid tracer-positron emission tomography) (n=5) and fulfilled the National Institute on Aging and Alzheimer's Association (NIA-AA) diagnostic criteria for MCI due to AD or AD dementia and were further classified into: amnesic (A-EOAD, 48 subjects) and non-amnesic variants (NA-EOAD, 22 subjects) (Albert *et al*., 2011; McKhann *et al*., 2011).
2) Autosomal dominant Alzheimer's disease (ADAD) (n=14): Seven with MCI and seven with mild dementia due to AD were retrospectively. All of them carried a pathogenic mutation in the *PSEN1* gene.
3) Sporadic FTD (n=25): Eleven subjects accomplished behavioral variant of FTD (bvFTD) diagnostic criteria, five of non-fluent variant for primary progressive aphasia (nfvPPA) and nine of semantic variant of primary progressive aphasia (svPPA) (Rascovsky *et al*., 2011; Gorno-Tempini *et al*., 2011). All FTD patients had normal AD CSF biomarkers results (n=22) or negative amyloid-PET (n=3).
4) Genetic FTD (n=7): Two subjects with *C9orf72* expansion (2 bvFTD), four with *GRN* mutation (three nfvPPA, one bvFTD) and one case with *MAPT* mutation (bvFTD).
5) Non-degenerative MCI (n=25): Subjects with MCI with normal AD CSF biomarkers and clinical features compatible with fibromyalgia, chronic fatigue syndrome or psychiatric disorders as anxiety or depression.
6) Controls (n=59) with no cognitive complaints and normal AD CSF biomarkers results. Forty-two controls age-matched with sporadic EOAD and FTD patients and 17 controls age-matched with ADAD patients.

CSF biomarkers determination

CSF levels of amyloid beta, total-tau, and phosphorylated-tau were measured using Innotest ELISAs following manufacturer's instructions (Fujirebio, Ghent, Belgium).

Brain MRI imaging

High-resolution sagittal T1-weighted images were acquired in a 3Tesla scan (Siemens Magnetom Trio, Erlangen, Germany) at the Magnetic Resonance Image Core Facility, using proprietary three-dimensional magnetization-prepared rapid-acquisition gradient echo: MPRAGE sequences (TR=2300 mseg; TE=2,98 mseg; acquisition matrix 256x256, voxel size 1x1x1).

Visual rating assessment

MRI visual rating assessment was performed by two clinical dementia experts (M.B, N.F) blind to all clinical information. They evaluated the following scales: anterior temporal scale (AT) (Davies *et al*., 2006), medial temporal atrophy (MTA) (Scheltens *et al.,*1992), posterior atrophy (PA) (Koedam *et al.,* 2011) and anterior atrophy scales including orbitofrontal (OF), anterior cingulate (AC) and fronto-insular (FI) regions (Davies *et al.,* 2009, Fumagalli *et al.,* 2014). All these scales were assessed for every subject following the previously described rating protocol (Harper *et al*., 2016).

Statistical analysis

Statistical analysis was conducted using Stata/IC 14.2 (College Station, Texas, USA). Categorical data was analyzed by χ^2 test and quantitative data by ANOVA and Student's *t*-test with Bonferroni *post-hoc* procedure. Inter-rater reliability of rating scales was determined by the intraclass correlation coefficient (ICC) by a two-way random, absolute for single-measures [ICC (2,1)] and average measures ICCs [ICC(2,k)]. The diagnostic accuracy was estimated using area under the curve (AUC) values of receiver operator characteristics. Balanced accuracy was calculated as 0.5 x (sensitivity + specificity).

Results

Demographics

Demographical data is shown in Table 1. The ADAD subjects and their paired controls were younger with respect to the other groups ($p<0.01$). Higher women rate was found in controls. No differences in disease duration between groups were found. EOAD and bvFTD had lower MMSE than controls as well as A-EOAD and bvFTD than non-degenerative MCI ($p<0.01$).

Inter-rater reliability of visual rating scores

The kappa index showed substantial agreement on all the scales. The ICC values for single and average measures were excellent (Supplementary Material Table 1).

Mean rating scores per diagnostic group

Detailed rating score data is summarized in Table 1 and Fig.1. A-EOAD had higher scores than controls in all scales ($p<0.05$) and in AC and FI scales than non-degenerative MCI ($p<0.05$). The NA-EOAD showed higher ratings than controls in PA, AT and OF ($p<0.05$) and higher rates on PA and AT ($p<0.05$) than non-degenerative MCI. In EOAD subgroups comparison, a tendency to higher PA score in NA-EOAD and higher MTA score in A-EOAD were found, although they did not reach the statistical significance. In ADAD, MTA ($p<0.05$) and PA ($p<0.01$) scores were higher than controls.

Compared to controls and non-degenerative MCI, bvFTD obtained higher rates in AC, FI, OF, AT and MTA ($p<0.05$) and svPPA in FI ($p<0.05$) and AT ($p<0.01$). No differences were found with nfvPPA. All scales were higher in genetic FTD ($p<0.05$).

The bvFTD and svPPA groups showed higher scores in MTA and AT ($p<0.01$) than EOAD groups. Genetic FTD had higher scores in FI, OF and AT ($p<0.05$) scales than A-EOAD, in the AC, FI ($p<0.01$) and AT ($p<0.05$) than NA-EOAD. The detailed distribution of rating scores is included as Supplementary Material Fig.1.

Rating scales diagnostic performance

Detailed diagnostic performances of scales for each group comparison are shown in Table 3. In A-EOAD, AC (AUC=0.80), followed by FI and MTA (AUC=0.77) showed good diagnostic accuracies. In NA-EOAD and ADAD, the PA scale was the best one (AUC>0.80). Otherwise, none of the scales showed an acceptable diagnostic accuracy for discriminating EOAD from FTD or non-degenerative MCI (AUC≤0.75).

Diagnostic performance in bvFTD was very good (AUC>0.90) for AT, OF and AC scales and good for FI, MTA and PA (AUC>0.75). In svPPA, diagnostic performance was very good (AUC>0.90) for AT and MTA scales and good (AUC>0.75) for FI and OF, whilst in nfvPPA, only the AC scale showed good diagnostic performance (AUC=0.78). All scales showed a very good (AUC>0.90) or excellent (AUC>0.97) diagnostic performance in genetic FTD.

Discussion

In our EOD cohort, the atrophy visual rating demonstrated to be reliable when based in a well-structured evaluation (Harper *et al*., 2016). The best scales for identifying AD and FTD from controls were different according to the clinical phenotype. The AC scale was the best for A-EOAD and PA scale for NA-EOAD, highlighting the little diagnostic accuracy of MTA in EOAD. Otherwise, AT is better to discriminate bvFTD and svPPA from controls as well as the AC scale for nfvPPA. All the scales had a very good diagnostic performance for genetic FTD.

Previous studies have investigated the diagnostic performance of brain atrophy scales in dementia being most of them focused on late-onset dementias and without biomarkers supported diagnosis (Duara *et al*., 2013; Ferreira *et al*., 2015; Ten Kate *et al*., 2017; Claus *et al*., 2017, Yuan *et al.*, 2019). Solely a recent study studied a pathologically-proven sample mostly focused on EOD but in advanced clinical stages (EOAD mean MMSE was 16.6±6.3) (Harper *et al*., 2016). Furthermore, another previous study with no biomarker-supported diagnosis found out better diagnostic performance of visual rating scales on moderate-severe AD cases compared to the mild ones (Yuan *et al.*, 2019). To our knowledge, our study is the first to compare the visual brain atrophy assessment

exclusively focused on EOD in the initial symptomatic stages (MCI and mild dementia, MMSE 23.3±3.5) in a well-characterized cohort with biological confirmation of the disease. We consider it of interest, since this is the timeframe when these subjects will seek medical advice, thus it is precisely then when biomarkers should be more important for establishing an accurate diagnosis. Additionally, often in clinical practice the diagnostic dilemma is not between AD or FTD and healthy controls but between the different neurodegenerative dementias (AD vs FTD) and non-degenerative cognitive impairment; for this reason, a group of non-degenerative MCI was also included in the evaluation.

FTD and AD showed higher atrophy scores than non-degenerative MCI and controls. Higher rates of atrophy were found on FTD being especially remarkable in the genetic cases, accordingly to recent data reported on a larger genetic FTD cohort (Fumagalli *et al.*, 2019). As expected, all FTD variants except the nfvPPA, showed a predominant atrophy pattern in those scales reflecting frontotemporal damage, and relatively lesser on posterior areas (i.e PA scale) (Rabinovici *et al*. 2017).

Different atrophy spreading patterns between AD phenotypes have been defined. While amnesic AD is characterized by early atrophy of the hippocampus and medial temporal lobes before spreading to neocortex, non-amnestic AD has relative sparing of the hippocampus and other regions as parietal areas are more afected (Phillips *et al*., 2018). In our study, a tendency of greater atrophy on PA scale in NA-EOAD and MTA on A-EOAD was found. This predominant posterior atrophy in non-amnestic forms compared to the typical amnestic ones agrees with their clinical phenotype (Phillips *et al*., 2018; Koedam *et al*., 2010).

Diagnostic performance was good or excellent in most scales for FTD variants, except for the nfvFTD. The good diagnostic accuracy achieved by the MTA scale in these FTD variants was especially notable, which contrasts with its lower discriminative capacity in EOAD (Harper *et al*., 2016; Falgàs *et al*., 2019). This finding is especially relevant for the amnestic phenotypes since it is expected to have more hippocampal atrophy and higher MTA ratings. In addition, the MTA scale was not able to discriminate AD from FTD and neither from the non-degenerative MCI. Taking into account that MTA is

considered a hallmark of AD and a biomarker in the current AD diagnostic criteria, it is important to note its low diagnostic performance in EOAD, as well as highlighting that it would not accomplish with the consensus for valid AD biomarker of at least 80% sensitivity and specificity (The Ronald and Nancy Reagan Research Institute of the Alzheimer's Association and the National Institute on Aging Working Group, 1999).

Although the anterior cingulate functions and their relation with AD remain understudied, previous reports described its volume loss in AD (Jones *et al*., 2006). It has been related to executive functions such as unawareness of memory deficits, flexible thinking and apathy (Amanzio *et al*., 2011). In this line, AC scale was the best for A-EOAD in our cohort, however, its diagnostic performance was still disappointing (66% sensitivity, 83% specificity).

The PA scale demonstrated to have the best diagnostic performance for NA-EOAD and ADAD. Since parietal atrophy is more common in AD atypical presentations and it is not characteristic of FTD, the PA scale was expected to obtain good diagnostic performance in NA-EOAD, but surprisingly it was not enough to meet the consensus of AD biomarkers criteria.

As far as none of the scales reached the AD biomarkers criteria, clinicians should be aware of over relying on the presence of atrophy at the first evaluation in a subject with early-onset cognitive impairment. On a broader view, we might reconsider the role of brain atrophy assessment in the current AD diagnostic criteria, defining whether it should be a supportive criteria for clinical diagnosis instead of being categorized as a biological AD biomarker in the early-onset patients. This would allow to weigh the added diagnostic value of visual atrophy assessment in comparison to other more specific AD neurodegenerative biomarkers (i.e: total and phosphorylated tau on CSF) which would be more in concordance of what is defined on the new AD research framework (Clifford *et al*., 2018).

The main limitation of our study is the relatively small sample size in some of the FTD subgroups, although, as far as we are aware this is the larger reported cohort focused on early stages of EAOD and FTD patients with biomarker-supported diagnosis.

In summary, even if we found differences in single visual scales scores between groups, they have shown little utility in the differential diagnosis of EOD in early stages. Furthermore, the results of the present study evidenced that none of the single scales met the requirements for being a valid diagnostic biomarker. Further studies in other well-characterized cohorts to evaluate single visual rating scales usefulness in early stages of EOD are needed to confirm our data.

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Competing interests:

Authors state that there are no conflicts of interest to disclose.

Table 1. Demographics and mean visual rating scores

	HC	A-EOAD	NA-EOAD	Ndg-MCI	bvFTD	svPPA	nfvPPA	Genetic FTD	Significant differences
n	42	48	22	25	11	9	5	7	NA
Age at scan, mean (SD)	58.5 (3.7)	59.5 (4.2)	57.4 (3.8)	59.6 (4.1)	60.9 (5.2)	60 (4.3)	61.2 (3.4)	59.2 (4.7)	None
Sex, (male) %	21.4	37.5	54.6	52	72.7	66.7	60	14.3	a, i*, v*, w*, m*, p, q*
Disease duration at scan	NA	3 (1.5)	1.9 (2.5)	3.9 (3.3)	3.2 (1.6)	2.5 (1.4)	1.4 (2.1)	2.3 (0.8)	None
MMSE	28.7 (1.2)	23 (3.6)	24.2 (3.6)	26.4 (2.5)	24.9 (3.3)	26.3 (3)	25.8 (2.5)	25.1 (3.5)	d*, e*, i*, n, m*
CSF $A\beta_{42}$, mean (SD)	906 (271)	384 (101)	435 (139)	880 (165)	1043 (211)	864 (369)	620 (90)	944 (189)	a*, b*, c*, d*, e*, f*, h*, i*, j*
CSF t-tau, mean (SD)	209 (59)	776 (311)	631 (330)	244 (102)	288 (93)	246 (49)	289 (121)	286 (185)	a*, b*, c*, d*, e*, f*, g*, i*, j*,y
CSF p-tau, mean (SD)	48 (12)	122 (70)	98 (28)	51 (15)	54 (19)	38 (7)	47 (15)	45 (12)	a*, b*, c*, d*, e*, f*, g*, i*, j*
Mean visual rating scores									
Orbito-frontal, mean (SD)	0.3 (0.4)	0.9 (0.7)	0.9 (0.5)	0.4 (0.5)	1.5 (1.1)	1.2 (1)	0.9 (0.9)	1.9 (1.1)	c, d*, i, m, n, q, t*, u
Anterior Cingulate, mean (SD)	0.4 (0.5)	1.2 (0.8)	1 (0.7)	0.6 (0.5)	1.8 (1.1)	1 (0.9)	1.3 (0.7)	2.3 (0.8)	d*, e, h*, k, m, n, p, t*, u
Fronto-insula, mean (SD)	0.5 (0.5)	1.2 (0.7)	1.1 (0.5)	0.7 (0.7)	1.7 (0.8)	1.5 (0.6)	1.1 (0.4)	2.3 (0.5)	c*, d*, e, h*, m, n, q*, r, s, *t, u
Anterior temporal, mean (SD)	0.4 (0.4)	0.9 (0.6)	0.9 (0.3)	0.6 (0.4)	1.8 (0.8)	1.8 (0.6)	0.7 (0.3)	1.8 (0.8)	a*, b*, c, d*, f*, g*, h, i, j, l, m, n, o, q*, r*, t*, u
Medial temporal, mean (SD)	0.4 (0.4)	1.1 (0.7)	0.7 (0.6)	0.7 (0.5)	1.9 (1.1)	2 (0.8)	0.6 (0.6)	1.6 (0.8)	a*, b*, d*, f*, g*, l*, m, n, o, r, t*, u
Posterior, mean (SD)	0.6 (0.6)	1.2 (0.7)	1.5 (0.6)	0.8 (0.5)	1.2 (0.6)	1 (0.6)	1.1 (0.1)	1.9 (0.6)	d, j, j, t*, u

	Younger HC	ADAD	Significant differences
n	17	14	NA
Age, mean (SD)	46.8 (8.5)	48.3 (8.4)	None
Sex	35.3	57.1	
Disease duration at scan		3.7 (2.5)	NA
MMSE	29.6 (0.5)	22.7 (3)	x*
CSF $A\beta_{42}$, mean (SD)	807 (221)	322 (177)	x*
CSF t-tau, mean (SD)	222 (70)	935 (871)	x*
CSF p-tau, mean (SD)	46 (9)	119 (94)	x*
Mean visual rating scores			
Orbito-frontal, mean (SD)	0.3 (0.4)	0.4 (0.6)	None
Anterior Cingulate, mean (SD)	0.5 (0.6)	1 (0.7)	None
Fronto-insula, mean (SD)	0.5 (0.5)	0.8 (0.7)	None
Anterior temporal, mean (SD)	0.4 (0.4)	0.5 (0.5)	None
Medial temporal, mean (SD)	0.2 (0.4)	0.6 (0.6)	x
Posterior, mean (SD)	0.4 (0.5)	1.1 (0.6)	x*

[a]Amnesic AD vs behavioral variant FTD
[b]Amnesic AD vs Semantic dementia
[c]Amnesic AD vs genetic FTD
[d]Amnesic AD vs controls
[e]Amnesic-AD vs non-degenerative MCI
[f]Non-amnesic AD vs behavioral variant FTD
[g]Non-amnesic AD vs Semantic dementia
[h]Non-amnesic AD vs genetic FTD
[i]Non-amnesic AD vs controls
[j]Non-amnesic AD vs non-degenerative MCI
[k]behavioral variant FTD vs Semantic dementia
[l] Behavioral variant FTD vs Non-fluent aphasia
[m] Behavioral variant FTD vs controls
[n] Behavioral variant FTD vs non-degenerative MCI
[o] Semantic variant FTD vs Non-fluent aphasia
[p] Semantic variant FTD vs genetic FTD
[q] Semantic variant FTD vs controls
[r] Semantic variant FTD vs non-degenerative MCI
[s] Non-fluent aphasia vs genetic FTD
[t] Genetic FTD vs controls
[u] Genetic FTD vs non-degenerative MCI
[v] Genetic FTD vs behavioral variant
[w] MCI vs controls
[x] Familiar AD vs younger controls
[y]Non-fluent-aphasia vs amnesic AD
*Indicates significance at $p<0.01$, otherwise $p<0.05$; NA = not applicable

Abbreviations: Ndg-MCI, non-degenerative MCI

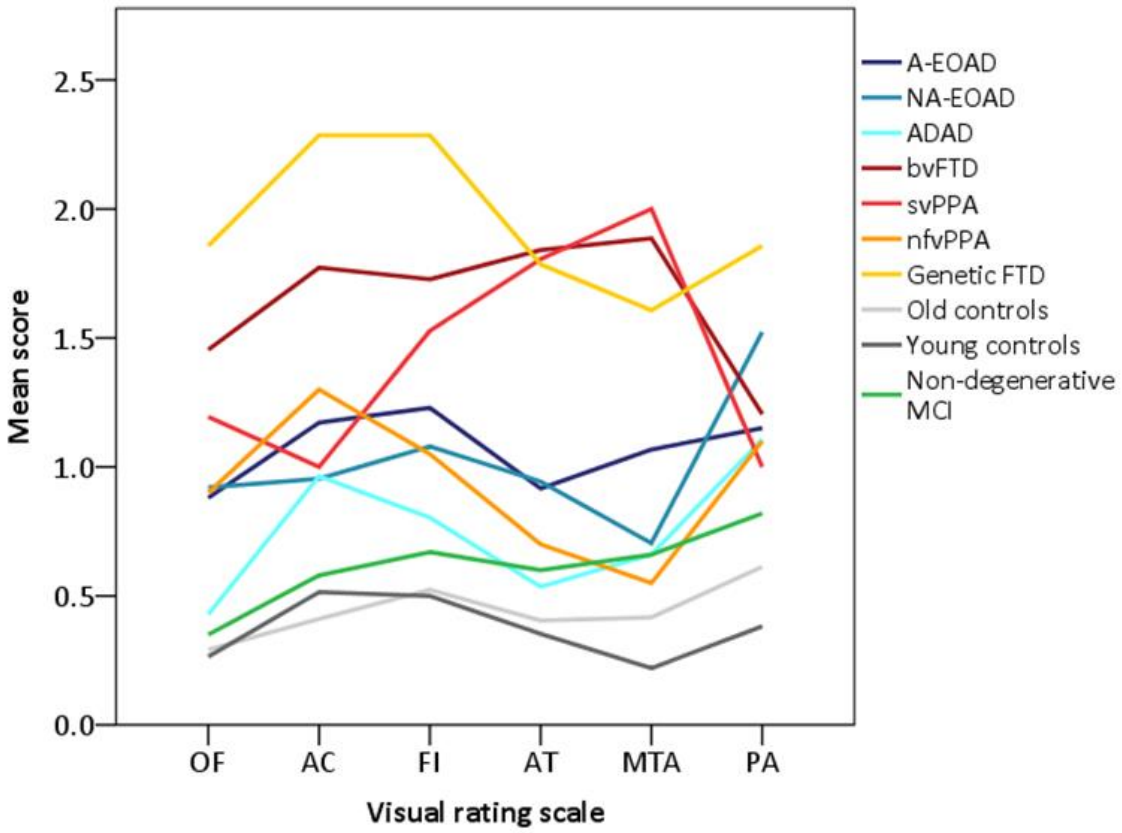
Table 2. Diagnostic performance of brain atrophy scales for each group comparison.

Classification task	**Scale** (cut-off)[a]	**Se**	**Sp**	**Balanced accuracy**	**AUC**
EOAD vs controls	AC (1)	67%	83%	75%	0.77
	FI (1)	71%	74%	73%	0.76
	OF (1)	60%	83%	71%	0.75
A-EOAD vs controls	AC (1)	66%	83%	75%	0.80
	FI (1)	71%	74%	72%	0.77
	MTA (1)	63%	81%	72%	0.77
NA-EOAD vs controls	PA (1.5)	68%	88%	78%	0.84
	AT (0.5)	95%	50%	73%	0.83
	OF (1)	68%	85%	77%	0.81
	FI (1)	68	86	73%	0.78
ADAD vs young controls	PA (1)	86%	69%	77%	0.81
	MTA (0.5)	64%	85%	74%	0.78
EOAD vs Ndg-MCI	None				
EOAD vs FTD	None				
FTD vs controls	AT (1.5)	84%	79%	81%	0.90
	FI (1)	84%	74%	79%	0.86
	MTA (1)	76%	81%	78%	0.84
	AC (1)	72%	83%	78%	0.83
	OF (1)	60%	86%	81%	0.80
bvFTD vs controls	AT (1)	91%	79%	85%	0.93
	OF (1)	64%	86%	75%	0.93
	AC (1)	82%	84%	83%	0.90
	FI (1)	91%	74%	82%	0.89
	MTA (1.5)	73%	98%	85%	0.88
	PA (1)	82%	60%	71%	0.75
svPPA vs controls	AT (1)	100%	79%	89%	0.98
	MTA (1)	89%	81%	85%	0.96
	FI (1)	89%	74%	81%	0.89
	OF (1)	67%	86%	76%	0.79
nfvPPA vs controls	AC (1)	80%	71%	78%	0.78
Genetic FTD vs controls	AC (1)	100%	83%	92%	0.97
	MTA (1)	100%	81%	90%	0.94
	AT (1.5)	71%	100%	86%	0.94
	FI (1)	86%	69%	77%	0.94
	PA (1.5)	86%	88%	87%	0.92
	OF (1.5)	57%	100%	79%	0.92

[a]Sensitivity and especificity of scales with AUC over 0.75 and balanced accuracy over 70% are shown. The optimal cut-offs for each scale are those with higher balanced accuracy. They should be interpreted as: < cut-off = normal, ≥ cut-off: abnormal.

Abbreviations: Se, sensitivity; Sp, specificity; Ndg-MCI, non-degenerative MCI

Figure 1. Distribution of mean rating scores for each group



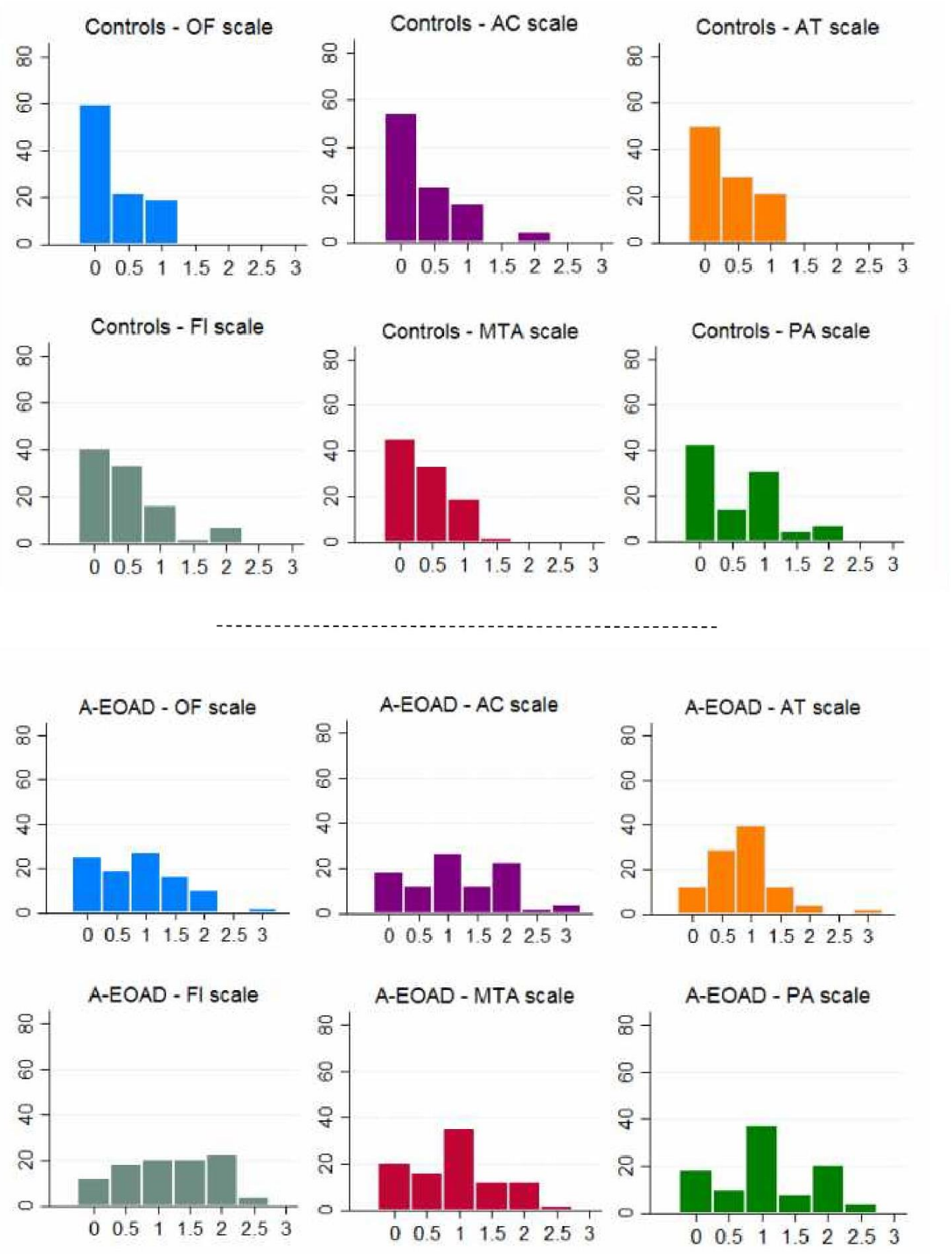
Supplementary Material

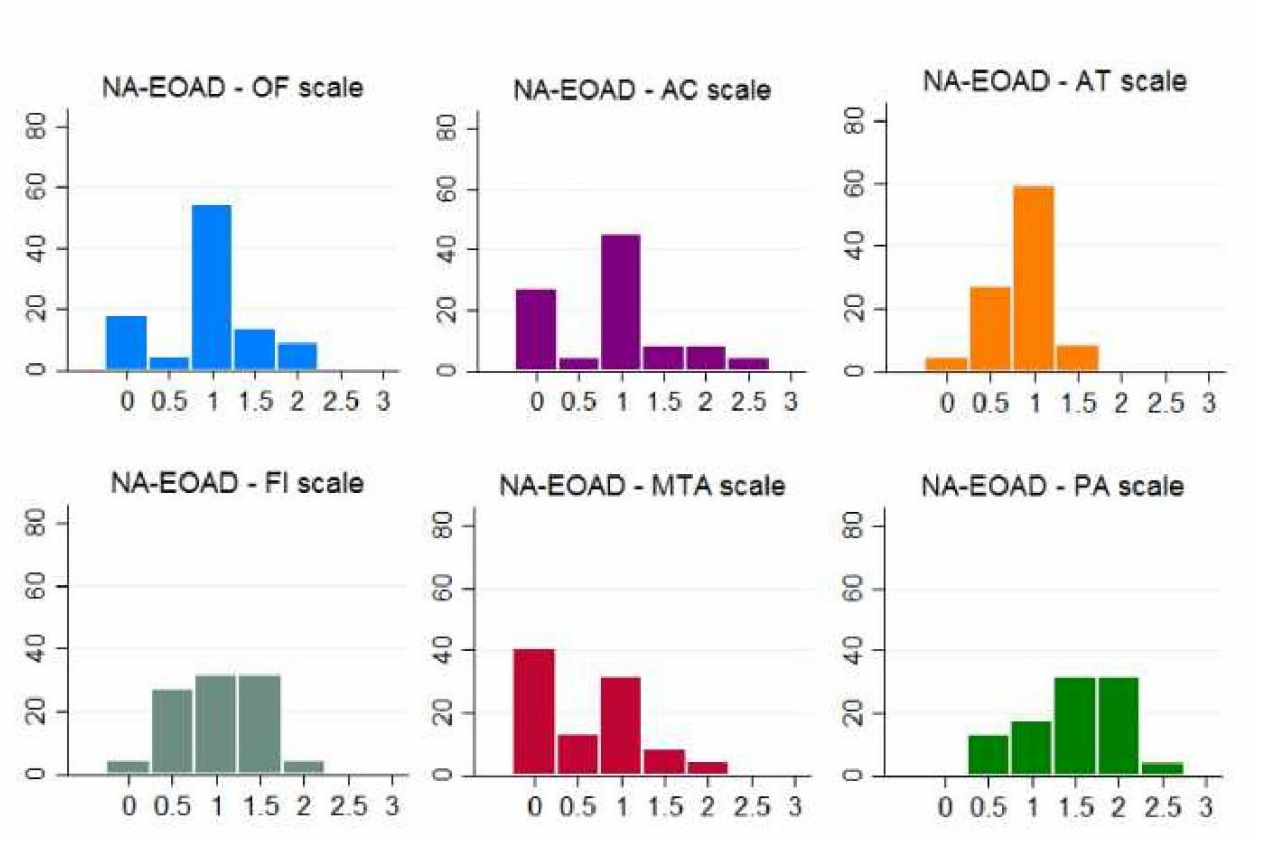
Table 1. Inter-rater reliability of visual rating scores

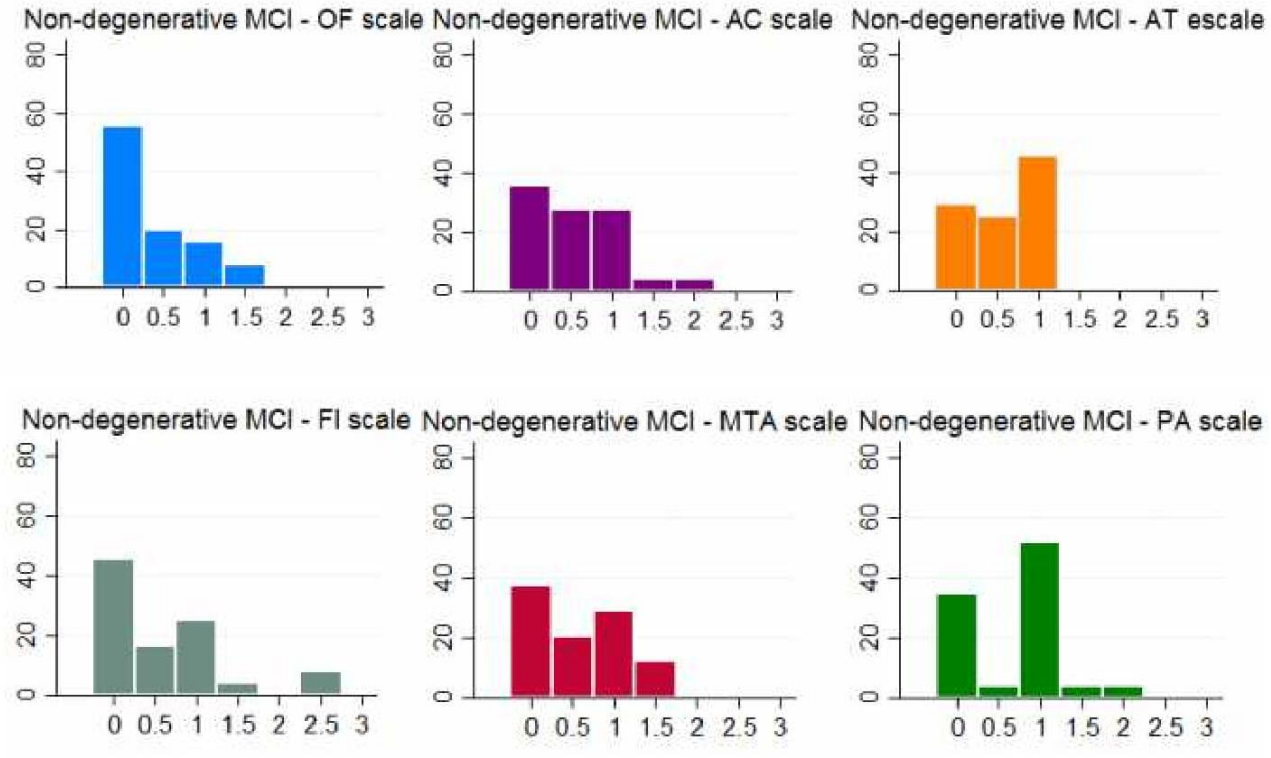
Scale	**Single mesures ICC** 2 rater *n*=200	**Average-Mesures ICC** 2 rater *n*=200
Left MTA	0.87 (0.82-0.90)	0.93 (0.90-0.95)
Right MTA	0.83 (0.75- 0.89)	0.91 (0.86-0.94)
Left PA	0.85 (0.80-0.89)	0.92 (0.89-0.94)
Right PA	0.84 (0.79-0.89)	0.92 (0.88-0.94)
Left AT	0.86 (0.81-0.89)	0.92 (0.90-0.94)
Right AT	0.89 (0.85-0.92)	0.94 (0.92-9.96)
Left OF	0.90 (0.87-0.92)	0.95 (0.93-0.96)
Right OF	0.91 (0.88-0.93)	0.95 (0.94-0.96)
Left AC	0.88 (0.84-0.91)	0.94 (0.91-0.95)
Right AC	0.91 (0.88-0.93)	0.95 (0.94-0.96)
Left FI	0.90 (0.87-0.92)	0.95 (0.93-0.96)
Right FI	0.89 (0.85-0.92)	0.94 (0.92-0 .96)

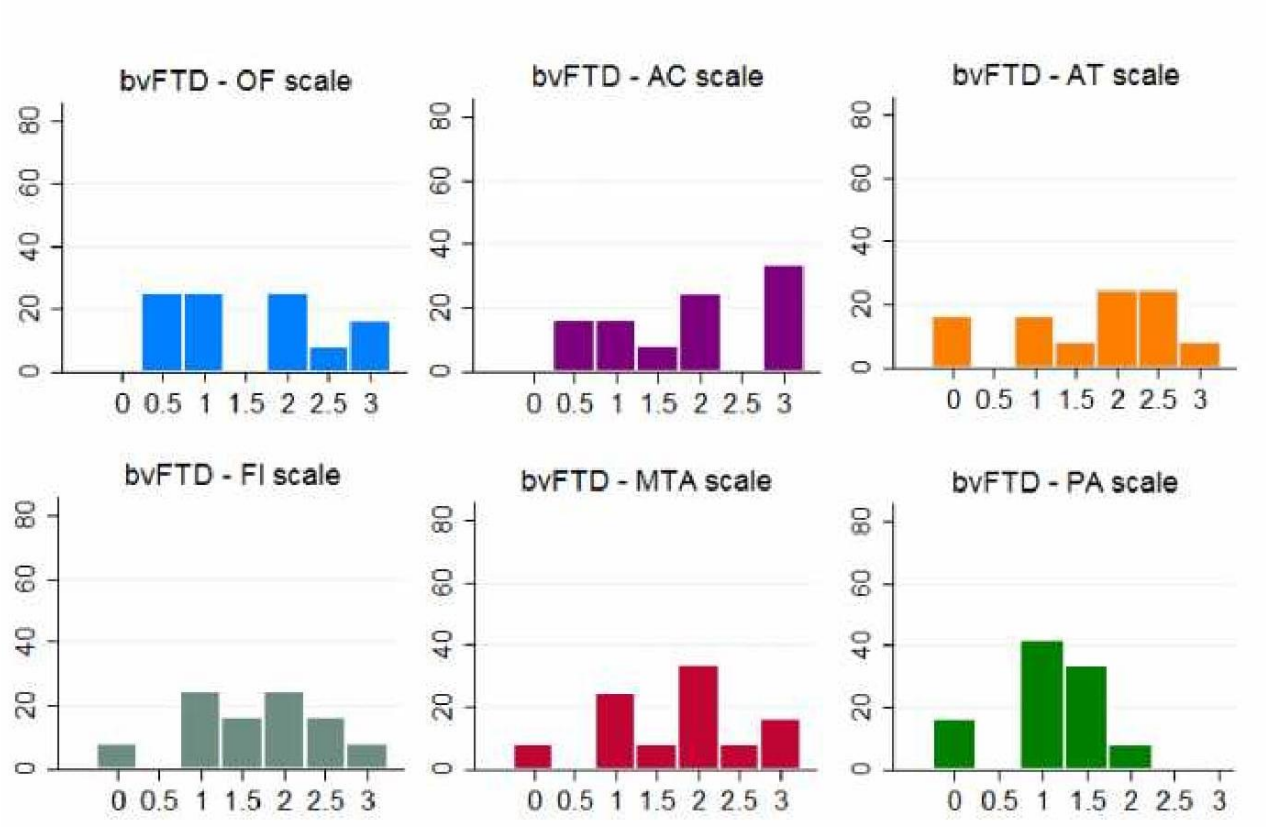
Scale	**Agreement** (%) 2 rater *n*=200	**Kappa** 2 rater *n*=200
Left MTA	81	0.71
Right MTA	76	0.63
Left PA	83.8	0.75
Right PA	84	0.75
Left AT	85.5	0.76
Right AT	87.5	0.79
Left OF	86.5	0.78
Right OF	87	0.79
Left AC	84	0.76
Right AC	88	0.82
Left FI	86.4	0.80
Right FI	85.5	0.79

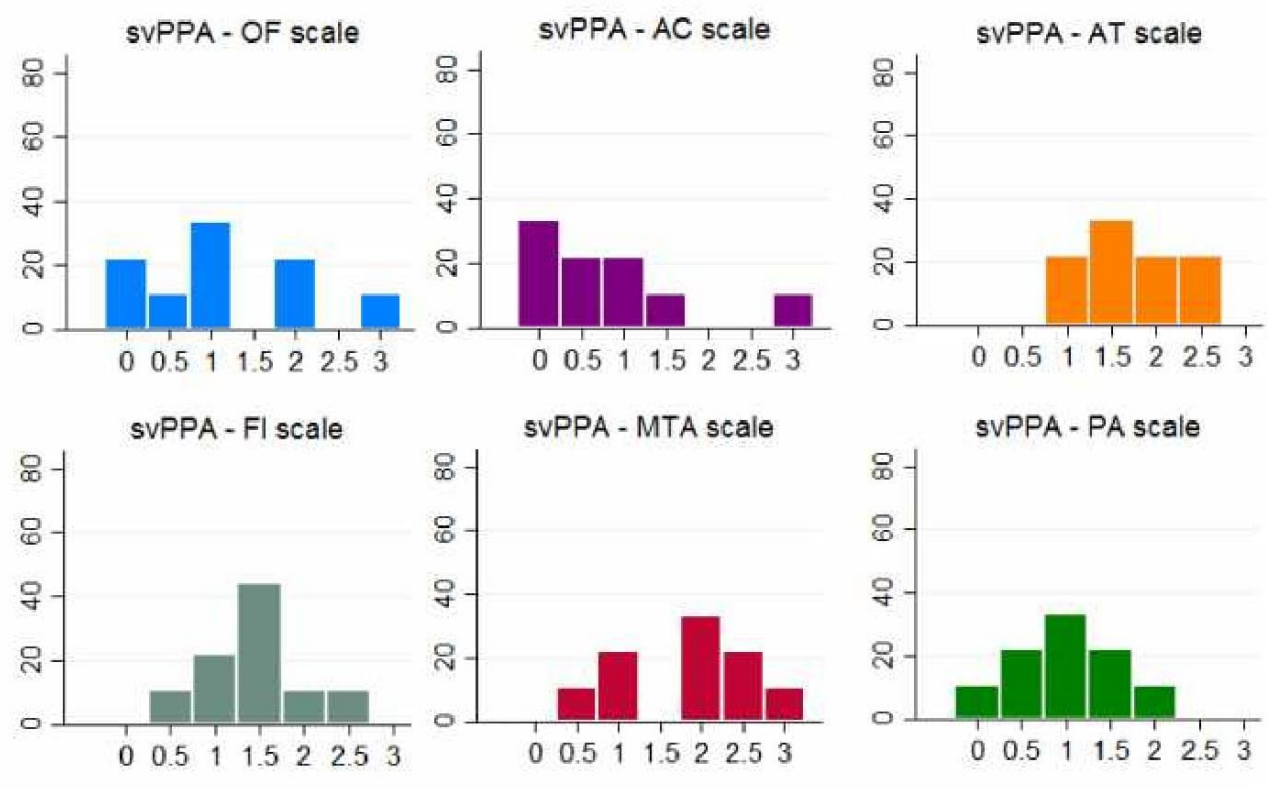
Fig. 1. Distribution of rating scores

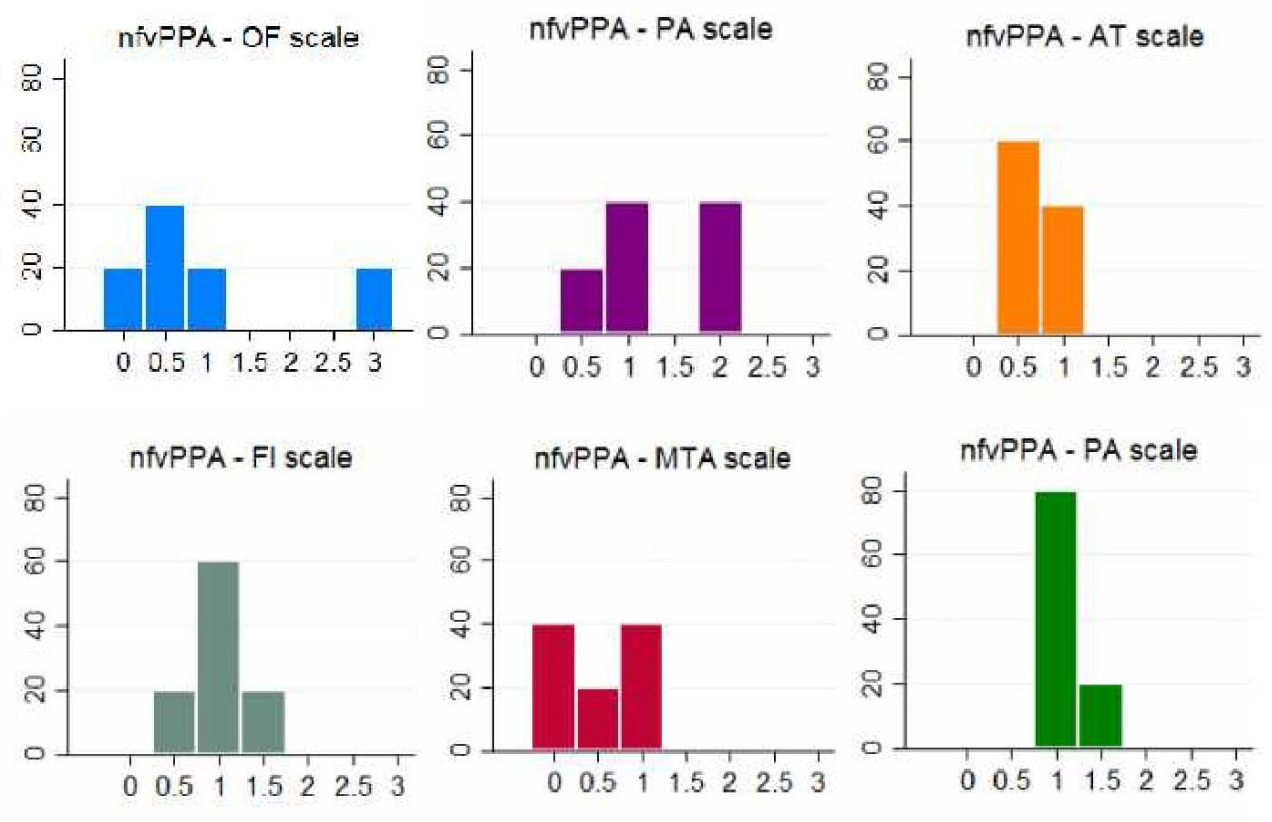


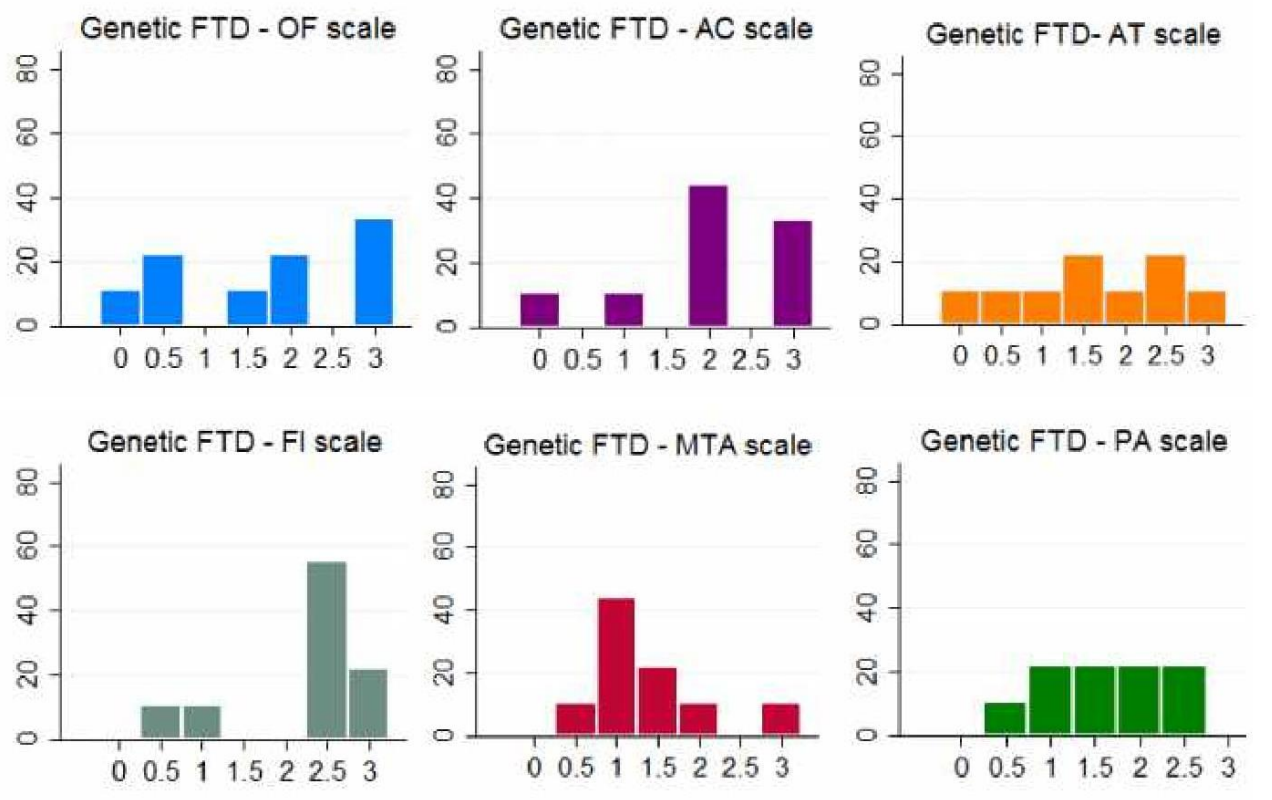

NA-EOAD - OF scale
NA-EOAD - AC scale
NA-EOAD - AT scale
NA-EOAD - FI scale
NA-EOAD - MTA scale
NA-EOAD - PA scale
0 20 40 60 80
0 0.5 1 1.5 2 2.5 3

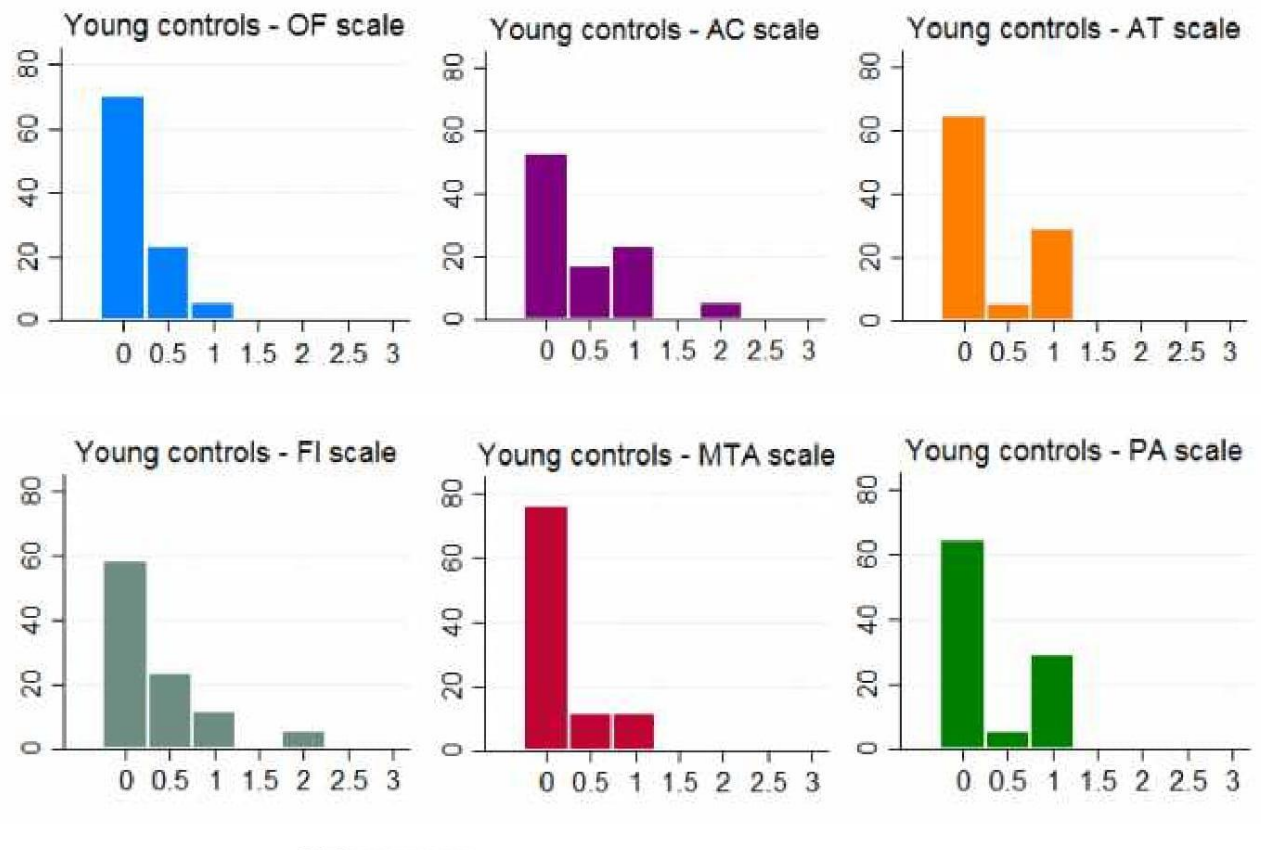

Non-degenerative MCI - OF scale
Non-degenerative MCI - AC scale
Non-degenerative MCI - AT escale
Non-degenerative MCI - FI scale
Non-degenerative MCI - MTA scale
Non-degenerative MCI - PA scale
0 20 40 60 80
0 0.5 1 1.5 2 2.5 3

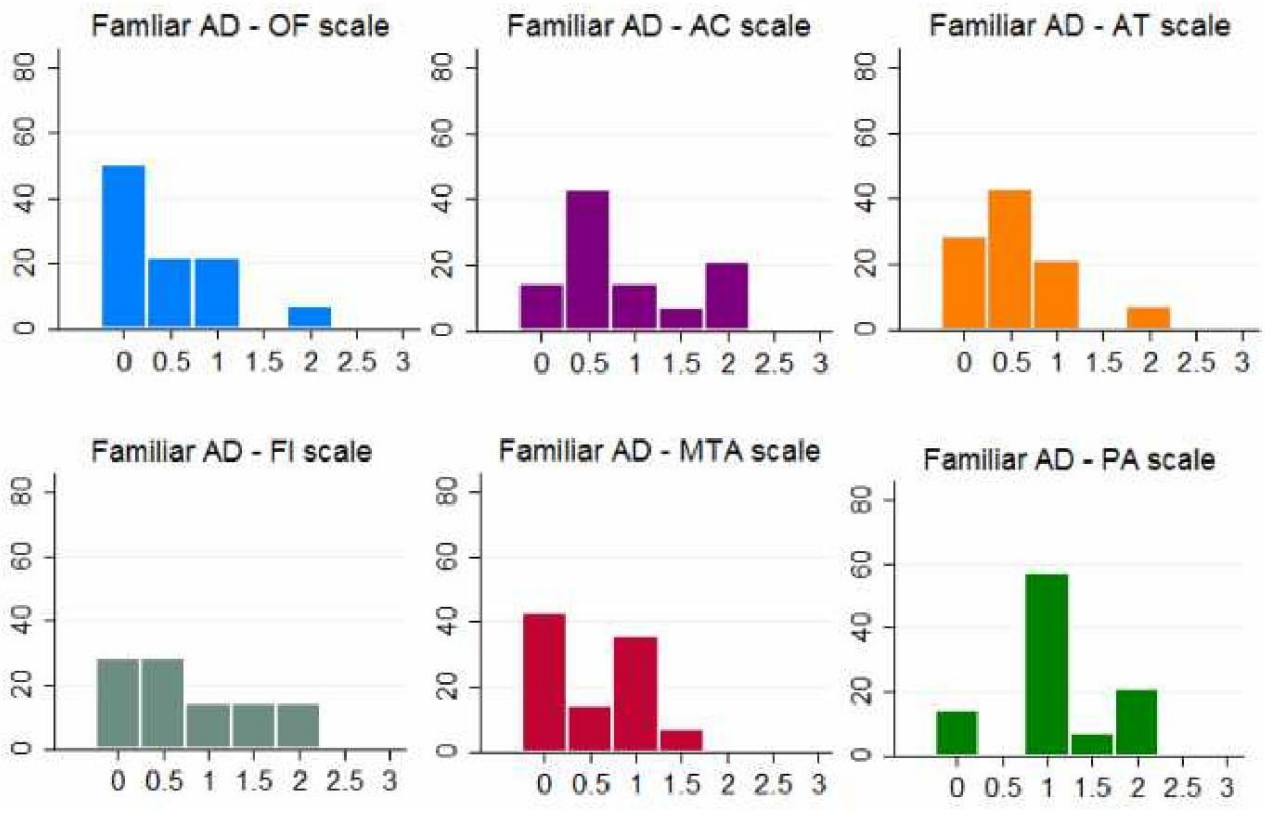

bvFTD - OF scale
bvFTD - AC scale
bvFTD - AT scale
bvFTD - FI scale
bvFTD - MTA scale
bvFTD - PA scale
0 20 40 60 80
0 0.5 1 1.5 2 2.5 3


svPPA - OF scale
svPPA - AC scale
svPPA - AT scale
svPPA - FI scale
svPPA - MTA scale
svPPA - PA scale
0 20 40 60 80
0 0.5 1 1.5 2 2.5 3


nfvPPA - OF scale
nfvPPA - PA scale
nfvPPA - AT scale
nfvPPA - FI scale
nfvPPA - MTA scale
nfvPPA - PA scale
0 20 40 60 80
0 0.5 1 1.5 2 2.5 3


Genetic FTD - OF scale
Genetic FTD - AC scale
Genetic FTD- AT scale
Genetic FTD - FI scale
Genetic FTD - MTA scale
Genetic FTD - PA scale
0 20 40 60 80
0 0.5 1 1.5 2 2.5 3


Young controls - OF scale
Young controls - AC scale
Young controls - AT scale
Young controls - FI scale
Young controls - MTA scale
Young controls - PA scale
0 20 40 60 80
0 0.5 1 1.5 2 2.5 3


Famliar AD - OF scale
Familiar AD - AC scale
Familiar AD - AT scale
Familiar AD - FI scale
Familiar AD - MTA scale
Familiar AD - PA scale
0 20 40 60 80
0 0.5 1 1.5 2 2.5 3

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Treball número 4:

Contribution of CSF biomarkers to early-onset Alzheimer's disease and frontotemporal dementia neuroimaging signatures

Falgàs N, Ruiz-Peris M, Sala-Llonch R, Antonell A, Balasa M, Borrego S, Ramos-Campoy O, Augé JM, Castellví M, Tort-Merino A, Olives J, Fernández-Villullas G, Blennow K, Zetterberg H, Bargalló N, Lladó A, Sánchez-Valle R

-Under Review-

Contribution of CSF biomarkers to early-onset Alzheimer's disease and frontotemporal dementia neuroimaging signatures

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Running title: CSF biomarkers profile for the AD and FTD signatures.

ABSTRACT

Background: Prior studies have described distinct patterns of brain grey matter (GM) and white matter (WM) alterations in Alzheimer's disease (AD) and frontotemporal lobar degeneration (FTLD), as well as differences in their cerebrospinal fluid (CSF) biomarkers profiles. We aim to investigate the relationship between early-onset AD (EOAD) and FTLD structural alterations and CSF biomarker levels.

Methods: We included 138 subjects (64 EOAD, 26 FTLD and 48 controls), all of them with a 3T MRI brain scan and CSF biomarkers available (the 42 amino acid-long form of the amyloid-beta protein [Aβ42], total-tau protein [T-tau], neurofilament light chain [NfL], neurogranin [Ng] and 14-3-3 levels). We used FreeSurfer and FSL to obtain cortical thickness (CTh) and fraction anisotropy (FA) maps. We studied group differences in CTh and FA and described the 'AD signature' and 'FTLD signature'. We tested multiple regression models to find which CSF-biomarkers better explained each disease neuroimaging signature.

Results: CTh and FA maps corresponding to the AD and FTLD signatures were in accordance with previous literature. Multiple regression analyses showed that the biomarkers that better explained CTh values within the AD signature were Aβ and 14-3-3; whereas NfL and 14-3-3 levels explained CTh values within the FTLD signature. Similarly, Aβ42, T-tau and age explained the FA values within the AD signature, while NfL and 14-3-3 levels explained FA values in the FTLD signature. Ng levels were not predictive in any of the models.

Conclusions: Biochemical markers contribute differently to structural (CTh and FA) changes typical of AD and FTD

1. INTRODUCTION

Early-onset dementia (EOD) is usually defined by a clinical onset under 65 and can reach up to the 5-10% of patients with dementia. Alzheimer's disease (AD) is the most common cause of EOD, followed by frontotemporal lobar degeneration (FTLD) (1). Early-onset AD (EOAD) is characterized by a faster disease progression and atypical presentations (non-amnestic symptoms) overlapping with other neurodegenerative dementias such as FTLD making the diagnosis more challenging (2,3). Thus, the use of neuroimaging and biochemical biomarkers is especially suitable in EOD in order to establish an early and accurate diagnosis (4).

Several studies have aimed to determine the different profiles of cerebrospinal fluid (CSF) biomarkers in different neurodegenerative diseases such as AD or FTLD (5). Some of these biomarker profiles have been well described whilst other novel biomarkers are still under investigation. Decreased amyloid-beta protein 42 (Aβ42) with increased total tau (T-tau) and phosphorylated tau (P-tau) levels define the typical AD biochemical profile (6, 7, 8, 9). Regarding novel biomarkers, neurofilament light chain (NfL) has been proposed as a non-specific neurodegeneration marker and while it is increased both in AD and FTLD as well in several other neurodegenerative disorders (i.e., amyotrophic lateral sclerosis or multiple sclerosis) (10, 11), CSF NFL has been shown to be of particular use for the differentiation between FTLD and early-onset AD while levels are higher in AD with onset in late life (12, 13). Neurogranin (Ng) is a synaptic (dendritic) marker that has been suggested to be specific for AD (14, 15), although increased levels are also found in Creutzfeldt-Jakob diseases with very intense neurodegeneration (16). The 14-3-3 protein has been extensively studied in Creutzfeldt-Jakob disease, but its participation on the AD neuropathological process has also been described (17, 18, 19, 20). Furthermore, previous studies have suggested that some of these biomarkers, as NfL or Ng, could provide information about the disease prognosis in AD and FTLD, respectively (21, 22, 23, 24).

Neuroimaging using Magnetic Resonance Imaging (MRI) has been widely used to describe cortical thickness (CTh) and white matter (WM) loss patterns in AD and FTLD as well as to find differential trajectories along the different disease stages (25, 26, 27, 28, 29, 30).

The relationship between AD neuroimaging features and classical AD biochemical markers and their reciprocal influence have been studied during both the clinical and preclinical phases of the disease (28). However, studying the influence of new biomarkers is more challenging as the trajectories have been poorly described and they might interact with those already reported, possibly giving non-trivial relationships. In this sense, how the different CSF biomarkers might explain or contribute to each disease atrophy pattern is still uncertain (31, 32).

In this context, our goals were 1) to provide a descriptive analysis of CSF-biomarker levels and structural patterns (CTh, hippocampal volume and FA) in early-onset AD and FTLD, 2) to study the relationship between early-onset AD and FTLD brain structural measures and CSF-biomarkers levels and 3) to perform a multivariate approach to evaluate which biomarkers better explained the characteristic structural alterations associated with each disease (i.e., disease signatures).

2. METHODS AND MATERIALS

2.1. Participants

One hundred thirty-eight subjects with disease onset under 65 were evaluated at the Alzheimer's disease and other cognitive disorders Unit at Hospital Clínic de Barcelona and were enrolled on this cross-sectional study. All subjects underwent a complete neurological and neuropsychological evaluation, 3T brain MRI scan and a spinal tap for the determination of CSF biomarkers. Subjects were classified into 3 groups:

- AD group (n=64): All EOAD patients fulfilled the National Institute on Aging - Alzheimer's Association (NIA-AA) diagnostic criteria for MCI due to AD or mild AD dementia and Mini-Mental State Examination (MMSE) ≥18 (8, 9). All subjects had a typical AD CSF biomarker pattern. Both early sporadic AD (n=52) and autosomal dominant AD (ADAD) (n=12) were included.
- FTD group (n=26): Ten behavioral variant of FTD (bvFTD) patients, 8 non-fluent variant for primary progressive aphasia (nfvPPA) and 8 semantic variant of primary progressive aphasia (svPPA) (33, 34) patients were included. Ten cases

were genetic cases (4 carried the *C9ORF72* expansion, 2 *MAPT* mutations and 4 *GRN* mutations). All FTLD were at mild phases of the disease (MMSE ≥ 18) at inclusion.

- Healthy controls (CTR) (n=48): healthy adults (age < 65 years old) with no cognitive complaints, cognitive performance within normative range and normal levels of AD CSF biomarkers.

The study was approved by the Hospital Clinic Barcelona Ethics Committee and all participants gave written informed consent.

2.2. CSF Biomarkers

Commercially available single-analyte enzyme-linked immunosorbent assay (ELISA) kits were used to determine levels of CSF Aβ42, T-tau and P-tau (INNOTEST, Fujirebio Europe N.V., Gent, Belgium), NfL (IBL International, Hamburg, Germany) (35) and 14-3-3 (CircuLex, MBL International Corporation, Woburn, MA, USA) (20) at the Alzheimer's Disease and Other Cognitive Disorders Unit Laboratory, Barcelona. CSF Ng concentration was measured using an in-house ELISA based on the monoclonal antibody Ng7 (epitope including amino acids 52-65 on Ng) as described previously (23). All 138 participants had CSF Aβ, T-tau, and P-tau levels available. NfL levels were available in 133 subjects, Ng in 104 and 14-3-3 in 94.

2.3. MRI acquisition

All participants were examined in the same 3T MRI scanner (Magnetom Trio Tim, Siemens Medical Systems, Germany). A high-resolution 3D structural data set (T1-weighted, MP-RAGE, repetition time = 2300 ms, echo time = 2.98 ms, 240 slices, field-of-view = 256 mm, voxel size = 1 x 1 x 1 mm) and a Diffusion Weighted echo-planar imaging (EPI) sequence (30 directions + b0 image, with two repeated acquisitions, TR = 7600 ms, TE = 89 ms, 60 slices, distance factor = 0%, FOV= 250 mm, matrix size =122 x 122, voxel size = 2 x 2 x 2 mm) were acquired for all subjects.

2.4. Cortical Thickness processing and analysis

CTh processing and vertex-wise statistical analyses were performed using FreeSurfer v6.0.0 (https://surfer.nmr.mgh.harvard.edu/). The entire pipeline is fully explained elsewhere (36, 37) and includes a set of methods applied to the T1-weighted MRI images to generate brain surfaces and CTh maps, calculated as the closest distance between the gray/white matter surface to the pial surface at each vertex of the tessellated surface (38). Before statistics, individual CTh maps were registered to a common space and smoothed using a FWHM of 15 mm.

Using the vertex-wise CTh data, we performed a set of analyses using general linear models (GLM). We first evaluated group differences, using age as covariate. Then, for each biomarker, we computed the correlation between the biomarker levels and CTh within each group. Results were corrected for multiple comparisons using monte-carlo simulations, and setting a threshold of $p<0.05$ for cluster significance.

2.5. Hippocampal volumes

Since the hippocampus is the main subcortical structure affected in AD and it is even considered an AD hallmark, we also assessed the hippocampal volume. We used the automated segmentation from FreeSurfer to obtain measures of hippocampal volume (39, 40). We calculated normalized hippocampal volume for each subject, by averaging left and right hippocampi and dividing by the total intracranial volume. We then calculated group differences and correlations with biomarker levels in hippocampal volume in R (https://www.r-project.org/.)

2.6. DTI processing and analysis

DTI processing and voxel-wise statistical analyses were performed with FSL v5.0.11 (41). Diffusion weighted images were first registered, using the B0 image as a reference volume, and corrected for motion and for eddy current effects. Then, non-brain tissue was removed using FSL's Brain Extraction Tool (BET) (42), and FA maps were obtained using the FMRIB Diffusion Toolbox. Tract-Based Spatial Statistics (TBSS) (43) was used for voxel-wise statistical analysis of FA maps. Basically, within the TBSS protocol, nonlinear transforms were first applied using FNIRT to obtain FA images aligned to standard space and the resulting images were merged into single 4D image. Then, the

mean of all FA images was fed into a skeletonization protocol obtaining the group mean FA skeleton. Finally, individual FA data were projected onto group skeleton.

DTI-based voxel-wise statistics on the FA maps were carried out using a permutation testing for nonparametric statistics using a GLM design. In a first GLM, we included the three main groups (CTR, AD and FTD), and we tested for differences between the three groups using age as a covariate. In a second set of analyses, we included individual biomarker values for each group, and subjects' age. We tested for correlations between FA and each biomarker in the three groups, using age as a covariate. This procedure was performed separately for NFL, T-tau, Aβ, Ng and 14-3-3.

2.7. Disease-specific signatures and multiple regression approaches

We created diseases signature maps, obtained from the group comparison analyses, namely CTh_{AD} and CTh_{FTD} and FA_{AD} and FA_{FTD}, for the CTh and FA maps. Signatures for CTh were obtained from the AD<FTD and the FTD<AD contrasts, in order to depict areas of specific cortical atrophy for each disease. Due to the fact that the direct comparison between AD and FTD in FA did not give significant results, we created FA signatures as the difference between the AD<CTR and the FTD<CTR maps.

After creating these disease-specific signature maps, we obtained individual CTh and FA values for each signature across the entire sample of subjects. We tested multiple regression models in order to evaluate the predictive capabilities of the different biomarkers for each signature, using Akaike Information Criterion (AIC) stepwise algorithm in R (44). For that, we used a sample of N=75 subjects, corresponding to those that had complete sets of MRI and CSF measures. Before multiple regression models, we evaluated pair-wise correlations of the different CSF biomarkers. We then created four separate models for predicting CTh_{AD}, CTh_{FTD}, FA_{AD} and FA_{FTD}, with Aβ, T-tau, NfL, Ng, 14-3-3 levels and age as predictors. We used 90% confidence intervals obtained with bootstrapping algorithms to study the significance of the models and the relative importance of each predictor. In addition, we evaluated the multiple regression models corresponding to the hippocampal volume and the MMSE results.

3. RESULTS

3.1. Sample Demographics, Clinical Data and biomarkers

Demographic information, MMSE scores and mean levels of the biomarkers are shown in Table 1. In summary, we found a slightly greater proportion of females in the CTR group compared with the FTLD group. FTLD subjects were slightly older than CTR and AD groups ($p<0.05$). MMSE scores were lower in both AD and FTLD groups compared with CTR ($p<0.05$), but did not differ between AD and FTLD. We found lower Aβ42 and higher T-tau and P-tau concentrations in AD compared to FTLD and CTR. Compared to CTR, NfL and 14-3-3 were higher in both AD and FTD, but in AD and FTLD comparison, NfL were higher in FTLD while 14-3-3 concentration was higher in AD. Ng levels in AD were higher than in CTR and FTLD (Table 1).

We found significant correlation between several pairs of biomarkers, both in the whole sample or in the different clinical groups (Supplementary Material Tables 1-4).

3.2. CTh results

3.2.1 Group differences in CTh

We found reduced CTh in frontal and temporal areas in FTLD compared with CTR, and widespread CTh loss in AD. We use the map resulting from the AD<FTLD contrast to represent the CTh_{AD} signature, and the reverse contrast for the CTh_{FTLD} (See Figure 1A).

3.2.2 CTh and CSF biomarkers correlation analysis

In FTLD, NfL levels showed a significant negative correlation with CTh in a cluster located on the left hemisphere (cluster size=15667.98 mm2, cluster p=0.0001), covering mainly frontal areas, including the pars opercularis, the pars triangularis, the middle and superior frontal and the precentral (Figure 2) gyrus. We also found a negative correlation between CTh and T-tau levels across several bilateral frontal areas, mainly the superior frontal gyrus (Figure 2). In AD, no correlations were found between biomarker values and CTh.

3.3. Hippocampal volumes: Differences across diseases and correlations with biomarkers

We found reduced normalized hippocampal volume in AD and in FTLD compared with CTR ($p=7.54\cdot10^{-10}$ and $p=4.51\cdot10^{-09}$ respectively). No differences in hippocampal volume were found between AD and FTLD (p=0.15).

When the three clinical groups were pulled together, normalized hippocampal volume showed correlations with Aβ42 (p=0.01, r=0.22 age-corrected), T-tau (p=0.046; r=-0.17), NfL (p=0.01, r=-0.23) and Ng (p=0.046, r=-0.20). We did not find any significant correlation between normalized hippocampal volumes and CSF biomarkers, when AD, FTLD and CTR groups were studied separately (all p>0.05).

3.4. DTI results

3.4.1 Group differences in FA

DTI analyses were performed with 112 subjects (49 AD, 23 FTLD and 40 CTR) with available DTI data of good quality. When comparing FA maps across groups, we found patterns of significantly reduced FA both for AD and FTLD vs CTR. These decreases were found generally across the entire skeleton for both diseases, with predominance in frontal areas and the left hemisphere in FTLD. When the two disease groups were compared, we found greater alterations in the left hemisphere in FTLD, whereas we could not detect areas with lower FA in AD. The signature patterns for FA were defined as the difference between the CTR>AD (FA_{AD}) and the CTR>FTLD (FA_{AD}) maps, both thresholded at corrected level of p<0.05. (Figure 1A)

3.4.2. FA and CSF biomarkers correlation analysis

In AD patients, we found a significant negative correlation between NfL values and FA in the forceps minor, the anterior thalamic radiation, the cingulum, the corticospinal tract, the uncinate fasciculus, the inferior longitudinal fasciculus, the inferior fronto-occipital fasciculus and the temporal part of the superior longitudinal fasciculus. In FTLD patients, FA values in the forceps minor, the anterior thalamic radiation, cingulum, forceps minor and the left superior longitudinal fasciculus correlated negatively with NfL (Figure 3). T-tau and 14-3-3 showed a negative correlation with FA in the forceps

minor for the FTLD group. The remaining biomarkers (Aβ, Ng) did not yield any significant results.

3.5. Disease signatures and multiple regression results

The areas within each signature, representing different patterns of structural damage in AD and FTLD, are described previously and shown in Figure 1B.

In the multiple regression analysis, we found that Aβ42 and 14-3-3 levels contributed to predict CTh levels within the CTh_{AD} area, explaining 28% of its variance, whereas CTh values within the CTh_{FTLD} area was better predicted by NfL and 14-3-3, explaining 29% of the variance. For FA values in $FA_{AD,}$ the predictive factors were Aβ, T-tau and age (29% of the variance), and for FA values in FA_{FTLD}, these were NfL and 14-3-3 (49% of the variance). Ng levels were not predictive in any of the models (Table 2).

In addition to the disease signature patterns, we created models for the hippocampus volumes (using the normalized bilateral volume) and for the MMSE scores. We found that Aβ42, NfL and AGE were the factors that better explained the hippocampal volume (28% of variance), whereas NfL, 14-3-3 and age, predicted the MMSE score (28% variance).

For each model, we calculated the relative importance of each predictor and we found that NfL had the highest impact in both the CTh values in CTh_{FTLD} (89%) and FA values FA_{FTLD} (92%), whereas Aβ had the highest importance for both CTh_{AD} (64%) and FA_{AD} (47%). The most important predictors for the hippocampal volume and for MMSE were NfL and 14-3-3 respectively (Figure 3B).

4. DISCUSSION

We performed a cross-sectional study of structural GM and WM alterations and their relationship with CSF biomarkers in early-onset AD and FTLD. Differential patterns of brain loss and CSF biomarkers profiles were found for both diseases. For the AD signatures, we found that, in addition to Aβ, 14-3-3 was the only neurodegeneration

marker that significantly contributed to CTh levels variation, whereas T-tau contributed to FA levels. For FTLD signatures, NfL and 14-3-3 were the main contributors to both CTh and FA values.

In our cohort, as expected, EOAD patients showed lower Aβ and higher T-tau and P-tau CSF concentrations compared to FTLD and controls (6, 7). NfL concentrations were higher in both diseases compared with CTR, and in FTLD compared to AD, in concordance with previous publications (12, 21, 22, 23, 45). Ng in AD were higher than controls and FTLD, but not significant differences were found in FTLD with respect to CTR (14, 15, 20, 43, 44, 45). There are few data about 14-3-3 levels in non-prion neurodegenerative disorders, in our cohort, 14-3-3 levels were increased in AD and FTLD compared with CTR and in AD compared with FTLD (17).

We found cortical and subcortical (hippocampus) GM loss in both AD and FTLD compared with controls. In general, the atrophy pattern was more widespread in AD and presented a fronto-temporal predominance in FTLD in line with previous publications (27, 49). We also found WM integrity loss in both diseases, greater in FTLD than AD. These findings are similar to previous studies evaluating the structural connectivity in neurodegenerative dementias that have suggested more WM damage in FTLD compared to AD, especially in frontal and temporal regions (25, 27, 50).

In the multivariate analysis, we found that AD and FTLD neuroimaging signatures were differentially influenced by CSF biomarkers. For AD, Aβ was the biomarker that most contributed to CTh and FA values within the AD signature. Unexpectedly 14-3-3 resulted a significant predictor of CTh values while other neurodegeneration markers as T-tau, NfL and Ng did not. T-tau and age, but not NfL also contributed to FA values. Previous studies have analyzed the contribution of Aβ and T-tau to structural changes in AD (16, 26, 53, 54, 55). In our cohort, 14-3-3 levels showed a strong correlation with T-tau levels both in the whole group and in the different clinical subgroups. This could suggest that

the effect of T-tau observed in other studies could be related to the 14-3-3 effect we observed in this study, while here the strong correlation observed could cancel the effect of T-tau in the regression model.

Both CTh and FA values within the FTLD signature were mostly explained by NfL levels, although 14-3-3 levels also contributed. NfL values were associated CTh and FA values in the left frontal and temporal regions in FTLD. These data support that NfL is a neurodegeneration marker strongly related to FTLD (42). These findings are also consistent with previous studies in FTLD patients that reported correlations between brain structure and NfL concentration especially in frontotemporal areas, with predominance in the left hemisphere (22, 24). The relation of NfL with WM changes, beyond the GM loss, is plausible as NfL is an axonal protein (51).
14-3-3 was also the main factor in MMSE, supporting a role as a non-disease specific marker of neurodegeneration. Regarding hippocampal volume, we found that Aβ and NfL accounted almost equally models suggesting both CSF biomarkers could contribute to the subcortical atrophy, as suggested previously (32).
Ng was the only CSF biomarker that did not influence any model, despite being altered in AD subjects even if has been suggested to be a specific biomarker of AD (56). Although our data further support previously reported elevated CSF Ng concentrations in AD compared with FTLD and controls, it did not reach relevance enough to outstand in the AD statistical model. These results are in line with a recent publication that reported that Ng did not show a diagnostic added value to the classic basic AD biomarkers in terms of diagnostic accuracy (47).

We should acknowledge several limitations in this study. First, the relatively small sample size, especially in the FTLD group. In this sense, the inclusion of different clinical FTLD variants can lead to some variability within the FTLD group, but which in turn reflects the heterogeneity of the FTLD itself. We also acknowledge that the fact that the maps obtained from the groups are then used in the analysis with biomarkers that also differ between groups could introduce some circularity. However, we think that the goal of evaluating which biomarkers better explained these structural changes is valid as we

use multiple regression models. Further studies in larger cohorts are needed to confirm and expand these data.

In conclusion, our study suggests that biochemical markers might contribute differently to structural (CTh and FA) changes typical of AD and these results support the complexity of the relationship between CSF biomarker and structural brain changes in these diseases.

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Disclosure statement:

HZ has served at scientific advisory boards of Roche Diagnostics, Wave, Samumed and CogRx, has given lectures in symposia sponsored by Biogen and Alzecure, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB, a GU Ventures-based platform company at the University of Gothenburg (all outside the submitted work). KB has served as a consultant or at advisory boards for Alector, Biogen, CogRx, Lilly, MagQu, Novartis and Roche Diagnostics, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB, a GU Venture-based platform company at the University of Gothenburg, all unrelated to the work presented in this paper.The other authors have nothing to disclose.

Table 1. Demographics, clinical data and CSF-biomarker values.

	Group means (SD)			Group comparisons		
	CTR	**AD**	**FTD**	CTR vs AD p value	CTR vs FTD p value	AD vs FTD p value
Gender (m/f)	14/34	28/36	14/12		0.036	
AGE mean (SD) years	54 (9.4)	57 (6.5)	60 (6)	0.106	$5.3*10^{-4}$	0.010
Aβ mean (SD) ng/L	788.02 (243.6)	397.8 (158.2)	812 (269.6)	$7.2*10^{-15}$	0.702	$4.4*10^{-8}$
P-Tau mean (SD) ng/L	49.3 (12.32)	115.42 (66.13)	49.4 (17.12)	$4.4*10^{-11}$	0.98	$1.2*10^{-10}$
T-Tau mean (SD) ng/L	215.79 (61.51)	857.36 (574.77)	331.36 (148.19)	$0.75*10^{-13}$	$6.6*10^{-4}$	$1.82*10^{-9}$
Nfl mean (SD) ng/L	378.31 (130.8)	1072.54 (537.2)	2687.3 (1530.6)	$3.3*10^{-15}$	$4.7*10^{-8}$	$1.4*10^{-5}$
14-3-3 mean (SD) ng/L	2461.8 (522.6)	5416.36 (2553.9)	3909.17 (1319.14)	$5.35*10^{-9}$	$7.01*10^{-5}$	0.0033
Ng mean (SD) ng/L	160 (55)	260 (130)	150 (62)	$5.35*10^{-6}$	0.28	$3.35*10^{-6}$
MMSE score	29.04 (1.11)	22.38 (4.85)	24.12 (4.86)	$3.9*10^{-16}$	$7.2*10^{-4}$	0.19

Descriptive data are of each group is presented as means±standard deviation. Statistically significant p values are shown in group comparisons. CTR, controls; AD, Alzheimer disease; FTD, Frontotemporal dementia; Aβ, amyloid-beta protein; P-Tau, phosphorylated-tau, T-Tau, Total-tau; Nfl, neurofilament light chain; 14-3-3, 14-3-3 protein; Ng, neurogranine; MMSE, Mini Mental state Examination.

Table 2. Biomarkers contribution to Alzheimer's disease and frontotemporal dementia signatures.

	Aβ	T-Tau	Nfl	Ng	14-3-3	AGE	R^2	Variance explained by model
CTh_{AD}	0.639 [0.193, 0.967]				0.361 [0.033, 0.807]		0.28	28%
FA_{AD}	0.466 [0.051, 0.889]	0.268 [0.013, 0.753]				0.266 [0.002, 0.642]	0.25	28%
CTh_{FTD}			0.890 [0.520, 0.999]		0.110 [0.001, 0.481]		0.31	29%
FA_{FTD}			0.924 [0.800, 0.995]		0.076 [0.006, 0.200]		0.47	49%
HV	0.365 [0.049, 0.688]		0.398 [0.039, 0.865]			0.237 [0.004, 0.637]	0.28	28%
MMSE			0.117 [0.0004, 0.584]		0.723 [0.217, 0.950]	0.160 [0.012, 0.406]	0.28	28%

These data show results of a multiple regression models. Coefficients are normalized to show relative contribution of each variable. CTh_{AD}/ $CTh_{FTD,}$: Mean cortical thickness values within the AD/FTD signatures; FA_{AD}/FA_{FTD}: Mean FA values within the defined FA/AD signatures. Aβ, amyloid-beta protein; P-Tau, phosphorylated-tau, T-Tau, Total-tau; Nfl, neurofilament light chain; 14-3-3, 14-3-3 protein; Ng, neurogranine; HV, hippocampal volumeMMSE, Mini Mental state Examination.

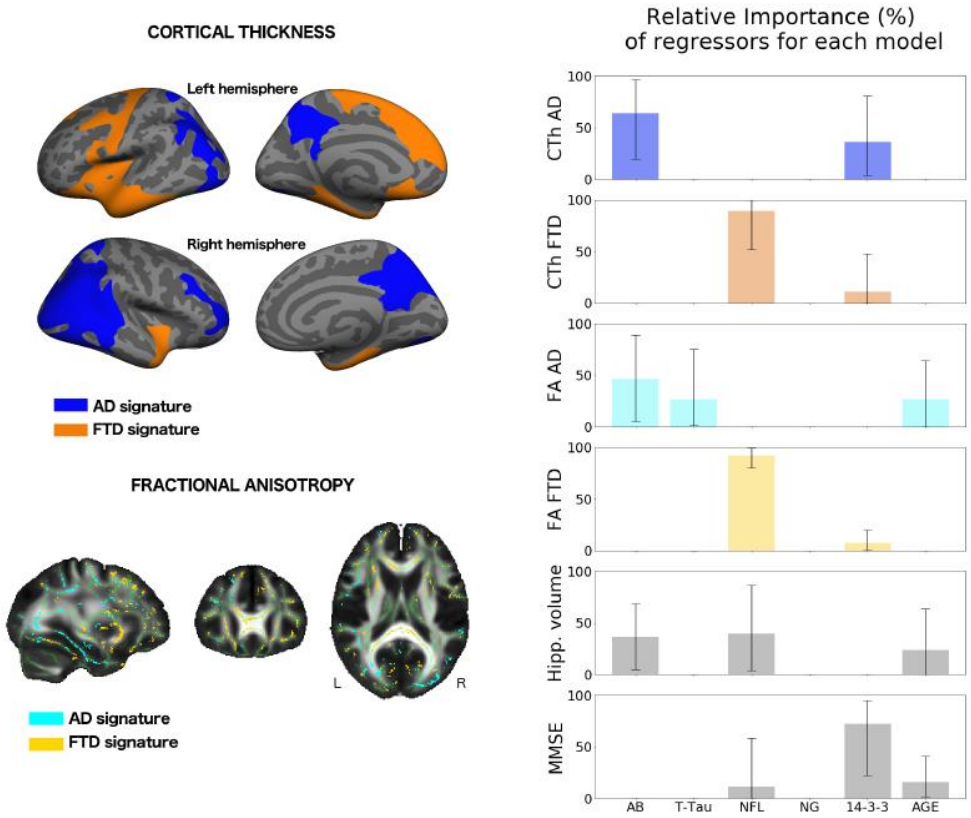

Figure 1. *(A) Patterns of specific structural alterations associated with Alzheimer's Disease (AD) and Frontotemporal Dementia (FTD). (B) Relative importance (%) of each CSF Biomarker and Age in each multiple regression model.*

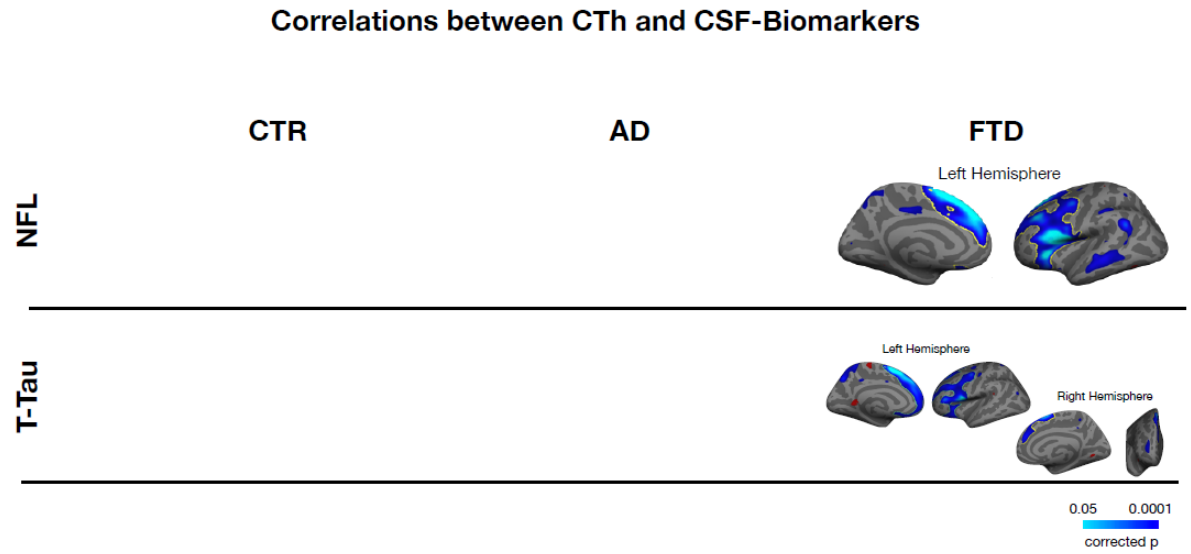

Figure 2. *Results of the vertex-wise correlations between CTh and the CSF Biomarkers. Significant correlations (corrected p<0.05) are shown in blue (negative correlations).*

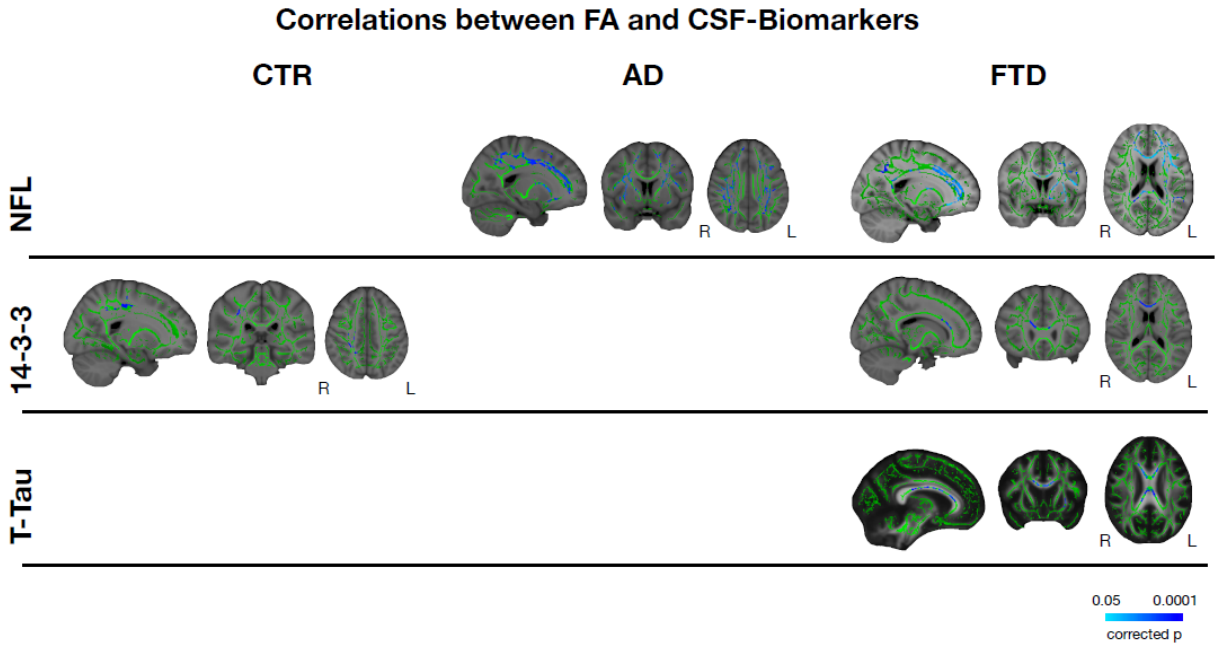

Figure 3. *Results of the voxel-wise correlations between FA and the CSF Biomarkers. Significant correlations (corrected p<0.05) are shown in blue.*

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Regional patterns of 18F-Florbetaben uptake in Presenilin 1 mutation carriers.

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Regional patterns of 18F-florbetaben uptake in presenilin 1 mutation carriers

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ABSTRACT

Individuals with autosomal dominant Alzheimer's disease (ADAD) present amyloid deposits before symptoms onset. We aimed to investigate efficacy and safety of 18F-florbetaben (FBB) for assessing amyloid deposition in ADAD. We acquired FBB positron emission tomography and magnetic resonance imaging of 25 individuals from *PSEN1* families (NCT02362880). We studied individual uptake patterns, group differences, and correlation with estimated years to symptoms onset, as well as adverse events. We found that asymptomatic carriers (N = 14) showed increased FBB uptake across the cerebral cortex and in the caudate. FBB accumulation appeared more than 15 years before onset in the precuneus and bankssts, among other regions, overlapping regions showing increased cortical thickness in the same subjects. FBB uptake correlated with estimated years to symptoms onset in several areas, especially the rostral anterior cingulate. Symptomatic carriers (N = 7) had an elevated FBB uptake *plateau*. No adverse events were reported. Overall, we found progressive FBB uptake in ADAD starting 2 decades before symptoms. The rostral anterior cingulate is a candidate area to track Aβ deposition in addition to the precuneus.

1. Introduction

Autosomal dominant Alzheimer's disease (ADAD), with almost 100% penetrance, allows the study of the trajectories of different biomarkers and the evaluation of disease-modifying treatments at early or presymptomatic stages of the disease (Bateman et al., 2011). In addition, the relatively predictable age of onset in ADAD allows the calculation of the estimated years to symptom onset (EYO), as a proxy of the distance to symptoms onset for each asymptomatic mutation carrier (MC). This facilitates the comparison between individuals with different mutations and the study of the progression of the disease even in cross-sectional samples. Using the EYO measure, research on ADAD, has revealed a temporal ordering of biomarkers indicating that the first pathological changes appear during the asymptomatic stage in target core regions and that these progressively expand toward new regions of the brain as the disease symptoms worsen. Brain amyloid-β (Aβ) accumulation is one of these biomarkers, and 2 ongoing trials, the Dominantly Inherited Alzheimer Network Trials Unit (Mills et al., 2013) and the Alzheimer's Prevention Initiative (Carrillo et al., 2013), are already testing drugs that aim to limit the amyloid accumulation in the brain in both ADAD and sporadic Alzheimer's disease (AD).

Positron emission tomography (PET) imaging is a reliable tool to explore the patterns of amyloid deposition. Different amyloid-PET tracers available have demonstrated accurate correlation with postmortem amyloid deposition. In ADAD, both the 11C-Pittsburgh

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compound B (PIB) (Bateman et al., 2012; Benzinger et al., 2013; Gordon et al., 2018; Quiroz et al., 2018; Rodriguez-Vieitez et al., 2016) and the 18F-florbetapir (Fleisher et al., 2012) tracers have been used, detecting elevated amyloid uptake about 15 years before estimated symptom onset. In addition, some of the patients with ADAD show relevant and early striatal amyloid tracer binding that is not frequent in sporadic AD (Benzinger et al., 2013).

However, it has not yet been established whether different amyloid tracers bind to identical sites on Aβ fibrils, offering the same ability to detect the regional Aβ burden. Regional and temporal disparities in fibrillar Aβ accumulation might have important implications for clinical trials. On the other hand, even if PIB-PET is considered the gold standard for amyloid tracers, its availability is more limited worldwide, given that it is a carbon tracer.

Florbetaben (FBB: trans-4-(N-methyl-amino)-4-{2-[2-(2-[18F] fluoroethoxy) ethoxy]-ethoxy} stilbene; Neuraceq) has high affinity and specificity for Aβ, lack of binding to Lewy bodies or neurofibrillary tangles in postmortem tissue at low nanomolar concentrations, and excellent correlation with global PiB retention (Rowe et al., 2017). However, no studies have been published to date examining the spatial pattern and chronology of 18F-florbetaben (FBB) binding in ADAD MCs. In this study, we investigate for the first time the efficacy and safety of the β-amyloid tracer FBB for assessing the regional and chronological amyloid deposition pattern in ADAD due to *PSEN1* mutations.

2. Material and methods

2.1. Participants

Participants were recruited within the genetic counseling program for familial dementias (PICOGEN) at the Hospital Clinic, Barcelona, Spain (Fortea et al., 2011). Participants were adult children (>18 years) of patients with symptomatic ADAD with a mutation in the *PSEN1* gene and who were either cognitively normal (Clinical Dementia Rating [CDR] = 0) or had mild symptoms of cognitive decline (CDR 0.5 or 1). Exclusion criteria were the presence of any condition precluding the completion of the study, any major medical disease, especially severe liver disease (aspartate aminotransferase/alanine aminotransferase levels > 5 × upper limits of normal) or advanced renal insufficiency (creatinine > 2 × upper limits of normal), current or previous history of alcohol abuse or epilepsy, known allergy to florbetaben or its constituents, pregnancy or breast feeding or planned pregnancy during the study period, and any medical or psychiatric disease other than ADAD, which could cause disturbance of brain function or cognition.

The study was approved by the hospital clinic ethics committee, and all subjects gave written informed consent. According to the Spanish regulations related to the use of radiotracers in human subjects, the study was registered as a clinical trial (EudraCT No. 2014-000763-41, protocol code FBB-FAD-2014, ClinicalTrials.gov Identifier: NCT02362880) and approved by the Spanish Drugs and Medical Products Agency.

Participants were classified as asymptomatic if they had no cognitive complaints, their cognitive performance was normal, and they scored 0 on the CDR scale, or as symptomatic if their CDR score was >0 or if their cognitive performance was ≥1.5 SD below the mean. In addition, according to their genetic status, participants were classified as MCs, either asymptomatic or symptomatic MC (AMC or SMC), and noncarriers or controls (CTR).

We calculated the EYO for AMC as the subject's age at the time of the study minus their parental age at symptoms onset. Furthermore, we divided the AMC into 2 groups: stage I-AMC, those younger than 15 years from estimated symptoms onset, and stage II-AMC, older than 15 years from estimated symptoms onset. In SMC, we calculated EYO as the subject's age at the time of the study minus their real age at symptoms onset.

2.2. Safety measures

Safety measures were recorded at the moment of the PET acquisition and after 24 hours. We recorded adverse events spontaneously reported by the participant, discovered as a result of general questioning by the study staff, or determined by physical examination, as well as findings of the medical and neurological examination and vital signs (blood pressure, heart rate, temperature).

2.3. MRI acquisition

Participants were examined on a 3T magnetic resonance imaging (MRI) scanner (Magnetom Trio Tim; Siemens Medical Systems, Germany). A high-resolution 3D structural data set (T1-weighted, MP-RAGE, TR = 2300 ms, TE = 2.98 ms, voxel size = 1 × 1 × 1 mm; FoV = 256 mm) was acquired. Two subjects (one SMC and one AMC) were acquired in a different scanner with comparable sequences.

2.4. PET acquisition

Each participant received a single intravenous bolus of approximately 300 MBq (8.1 mCi) of [18F] florbetaben, followed by a saline flush. Eighty minutes after injection, participants were scanned in a Siemens Biograph molecular CT. The scanning included a low-dose CT (50 mA, 120 kV) for attenuation correction and a PET acquisition consisting in 4 5-minute dynamic 3D frames. PET images were corrected for attenuation, death time, dispersion, and decay and reconstructed using an iterative algorithm (TRUEX+Time of Fly; 8 iterations, 21 subsets, and a gaussian with postfilter full width at half maximum, FWHM, of 3 mm), resulting in the final sequence of whole brain images of 109 planes (1.02 x 1.02 x 2.0 mm). Summed images were subsequently created for quantification and analyses.

2.5. Visual analysis of PET images

The visual pattern of tracer retention was evaluated by 2 physicians (authors NF and FL) blinded to the genetic and clinical status of the participants in the following areas: lateral temporal, frontal, parietal, posterior cingulate, precuneus, occipital, striatum, and cerebellum. Interpretation of the images was made comparing activity of gray matter with activity in adjacent white matter. FBB uptake in each of these regions was classified as pathologic/positive or nonpathologic/normal. Furthermore, cortical retention was classified as focal retention, if it was localized in a region, or as generalized uniform uptake, if it covered several regions of the cortical mantle.

2.6. MRI analysis

Images were processed with FreeSurfer (http://surfer.nmr.mgh.harvard.edu/), version 6.0. The FreeSurfer stream includes cortical reconstruction and registration, subcortical segmentation (Fischl et al., 2002), delineation of pial and white matter surfaces, estimation of the cortical thickness (CTh), and parcellation of the cortical surface (Fischl et al., 2004).

For whole-head segmentation of MRI data, we used an atlas specifically defined for PET analysis, consisting of 101 regions of interest (ROIs) covering cortical and subcortical areas (Greve et al., 2016). We performed visual validation of the surface reconstruction

and segmentation results. We calculated the mean CTh value within each cortical ROI of the atlas.

2.7. Automated PET quantification and analysis

Each individual PET scan was first registered to its corresponding MRI image using normalized mutual information (Greve and Fischl, 2009). All registrations were visually inspected.

We obtained the standardized uptake value ratio (SUVR) for each of the atlas ROIs, calculated as the mean uptake in the ROI divided by the uptake in the region of reference. We used the pons as a reference region according to previous studies (Su et al., 2016).

PET values were corrected for partial volume effects using the geometrical transfer matrix method (Greve et al., 2016). To reduce the number of measures, we calculated the bilateral mean (average between left and right) of the uptake in the main regions, resulting in 40 summary values per subject (6 subcortical and 34 cortical regions).

We used a previously reported cutoff of 0.78 (Bullich et al., 2017) to calculate the number of subjects with high FBB uptake at each region within each group. However, this threshold was obtained with results from a slightly different methodology based on ROIs from the automated anatomical labeling (AAL) atlas (Tzourio-Mazoyer et al., 2002). We used this threshold, as one of the most restrictive thresholds reported in the mentioned study, to account for changes in the methodology (FreeSurfer-based analysis with PVC vs. AAL-based analysis with no PVC). In addition, to further explore these differences between methods, we obtained the quantification results with the AAL-based analysis. These methods, as well as the comparison between results from the 2 approaches, are reported in Supplementary Material.

2.8. Statistical analyses

We compared the SUVR values between groups, using nonparametric tests in MATLAB. Specifically, we used Kruskal-Wallis and Mann-Whitney U for testing differences between groups of subjects. For each region, we tested 4 hypotheses: (1) higher uptake in SMCs than CTR; (2) higher uptake in AMCs than CTR; (3) higher uptake in stage I-AMCs than CTR; and (4) higher uptake in stage II-AMCs than CTR. We also used Spearman correlations to assess the association between individual SUVR scores and EYO, calculated in all MC and in AMC and SMC subjects separately.

In regard to CTh, we also performed group comparisons and correlation with EYO in the 34 cortical bilateral parcellations. All tests were considered significant at the $p < 0.05$ level. We calculated differences after correction for multiple comparisons using the false discovery rate (FDR) correction, for each hypothesis and across all the regions evaluated, implemented in MATLAB (Benjamini and Hochberg, 1995), with an FDR of $q<0.05$.

3. Results

3.1. Sample characteristics

Twenty-five participants from ADAD families caused by 12 different PSEN1 mutations (E120K, H131R, V151M, H163R, S169P, L173F, G209E, K239N, L282R, L286P, G378R, and I439S) were included. Of them, 14 were AMCs, 7 were SMCs, and 4 CTR. Demographic and clinical data are shown in Table 1. We observed group differences in age (Kruskal-Wallis test $p < 0.05$). CTR and AMCs differed in EYO (Mann-Whitney U, $p < 0.01$), but not in age. In our sample, we observed a bias toward female participants because of the fact that women from our ADAD clinical cohort were more prone to participate in research studies than men. Seven AMCs were classified as *stage I-AMCs*, younger than 15 years from estimated onset (mean EYO = −18.8 years, SD: 3.16), and 7 AMCs were classified as *stage II-AMC*, older than 15 years from estimated onset (mean EYO = −7.95 years, SD: 7.14).

3.2. Safety results

No adverse events were reported related to the tracer injection or PET scanning.

3.3. Visual assessment of PET images

Sixteen MCs, all 7 SMCs and 9 of 14 AMCs, showed abnormal (increased) FBB uptake at visual assessment at least in one of the areas studied. Seven of them (43.75%) showed both generalized uniform uptake and local striatal tracer uptake. They corresponded to 4 SMCs and 3 AMCs with EYO range −13 to 9.4 years and the following mutations: S169P, L173F, E120K, H163R, K239N, and L173F. Two L282R AMCs (12.5%) presented only generalized uniform cortical uptake. Two SMCs (12.5%) with EYO 3.1 and 4 years and G209E and H163R mutations showed only striatal retention. Four AMCs (25%) had focal cortical retention, with EYO range to −20.2 to −1.8 years and the K239N, L282R, G378R, and V151M mutations. One SMC (6.25%) with EYO 7.3 and L286P mutation presented cerebellar tracer retention in addition to generalized uniform cortical uptake. This participant was the only SMC who did not show striatal uptake at visual inspection. The 9 participants with high striatal uptake were 6 SMCs and 3 AMCs. The 5 remaining AMCs (EYO range: −24.4 to −15.7) were negative in all the areas evaluated at visual inspection. Fig. 1 provides one example of each of the amyloid patterns observed. All noncarriers were negative at visual inspection.

Table 1
Participant demographics and clinical variables

Variable	CTR	AMC	SMC	*p*
N = 25	4	14	7	
Age, y, mean (SD)	48.37 (10.01)	37.11 (8.79)	51.88 (2.32)	0.005
Sex, M/F	1/3	1/13	5/2	0.01
EYO, y, mean (SD)	2.06 (4.56)	−13.38 (7.73)[a]	4.88 (2.58)	<0.001
MMSE, mean (SD)	30	28.86 (1.56)	22.43 (3.31)	< 0.001
CDR total, mean (SD)	0	0	1.07 (0.67)	< 0.001
CDR-SOB, mean (SD)	0	0	4.57 (3.62)	< 0.001

p values obtained by Kruskal-Wallis test.
Key: AMC, asymptomatic mutation carrier; CDR, Clinical Dementia Rating; CTR, noncarriers or control; EYO, estimated years to symptoms onset; SMC, symptomatic mutation carrier; SOB, sum of boxes; SD, standard deviation.
[a] indicates that there are differences between AMCs and CTR.

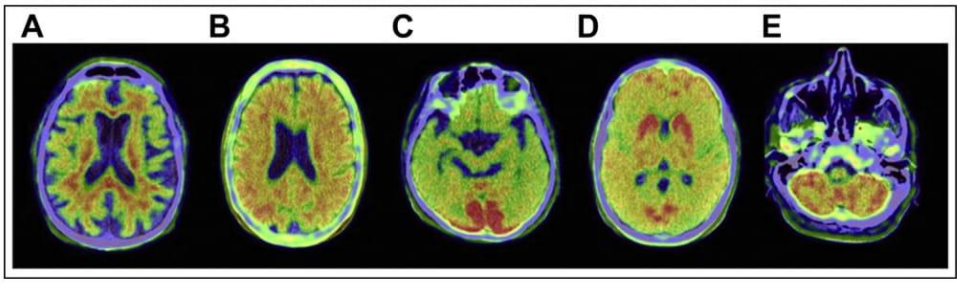

Fig. 1. Visual patterns of 18F-florbetaben (FBB) uptake observed in the PSEN1 mutation carriers. (A) Negative scan. (B) Generalized uniform uptake. (C) Focal occipital uptake. (D) Striatal and focal cortical uptake. (E) Cerebellar uptake.

3.4. Individual quantification

We studied the individual FBB uptake quantifications in all the regions. The percentages of subjects with altered (above cutoff) values within each group and for each region are summarized in Fig. 2. All SMC subjects showed regional SUVR values above the cutoff in all the areas studied except for the caudate (85.7% positive subjects), putamen (85.7% positive), hippocampus (14.2% positive), amygdala (57.4% positive), entorhinal cortex (0% positive), parahippocampal cortex (57.14% positive), and temporal pole (71.43% positive). Interestingly, the only SMC (EYO= 7.32 years, mutation L268P, and CDR = 1) subject that did not show increased SUVR in the caudate showed increased SUVR in the cerebellum. The youngest AMC who showed FBB above cutoff had an EYO of −20 years (7 out of 40 positive regions), and the second youngest had an EYO of −16 years (20 of 40 positive regions).

Of the stage I-AMC subjects, only the 2 subjects mentioned above, showed SUVR above cutoff in some areas: bankssts, precuneus, inferior and superior parietal, and supramarginal and lateral occipital (Fig. 2). However, all the *stage II*-AMC subjects presented at least one area with SUVR above the cutoff. CTR subjects present normal SUVR values in all the areas except for the pallidum.

The results obtained using the original methods defined in Bullich et al. (2017) are included in Supplementary Material, together with the comparative analysis between both sets of results. In summary, we found a strong correlation between both sets of measures and we obtained similar results both for group comparison analyses and in the rate of positive/negative subjects per region. We also tested the regional variability of the thresholds, and we concluded that the number of subjects classified as positive or negative was almost identical when using regional *vs* global thresholds (See Supplementary Material for results and further discussion).

3.5. Group differences in SUVR

In SMCs, we found higher FBB uptake in all the regions, compared with CTR (all surviving FDR threshold: $p = 0.023$, Fig. 3). In AMCs, the caudate and all cortical areas, with the exception of the entorhinal cortex, showed increased SUVR compared with CTR (all surviving FDR threshold: $p = 0.019$, Fig. 3). When AMC subjects were divided according to EYO, we found increased uptake ($p < 0.05$, uncorrected) in stage I-AMCs compared with CTR in 21 of 40 regions including 20 cortical ROIs and the caudate. However, none of these differences survived FDR correction. In stage II-AMCs, compared with CTR, we also found higher uptake in the hippocampus and in the rest of cortical areas (all surviving FDR threshold: $p = 0.037$, Fig. 4A and Supplementary Material).

3.6. Correlation between SUVR and EYO

For all MCs, we observed significant correlations between SUVR and EYO in all the areas studied (all surviving FDR threshold: $p = 0.0023$, see values in Supplementary Material). In AMCs, we found correlations between SUVR and EYO in many cortical areas and in the amygdala (all surviving FDR threshold: $p = 0.035$), which were not seen in SMCs. The regions with the strongest significant correlations ($r > 0.62$, $p < 0.05$, FDR corrected) are shown in Fig. 5.

3.7. Cortical thickness

In AMCs, we found higher CTh than CTR in the bankssts, paracentral, pars opercularis, pars triangularis, rostral middle frontal, and superior temporal ($p < 0.05$, Fig. 4B). In SMCs, we found thinner cortex than CTR in the precuneus and inferior parietal, superior parietal, and supramarginal regions (all $p < 0.05$, Fig. 4B). We found negative correlation with EYO in MCs in the bankssts, fusiform, inferior parietal, middle temporal, postcentral, precuneus, and supramarginal. We observed that all the areas mentioned previously, with significantly increased CTh in AMCs or decreased CTh in SMCs, showed also significant Aβ deposition compared with CTR (Fig. 4).

4. Discussion

We examined the spatial pattern of FBB retention in ADAD due to *PSEN1* mutations. Consistent with the amyloid hypothesis and

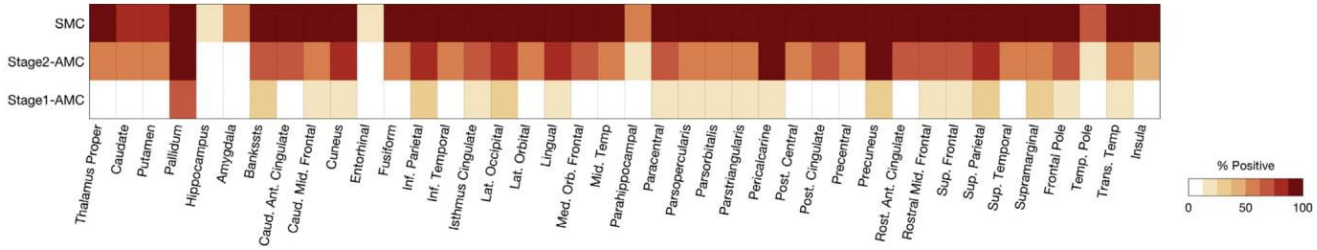

Fig. 2. Percentage of subjects with increase in individual standardized uptake value ratio (SUVR) values (cutoff = 0.78) for each region at each stage.

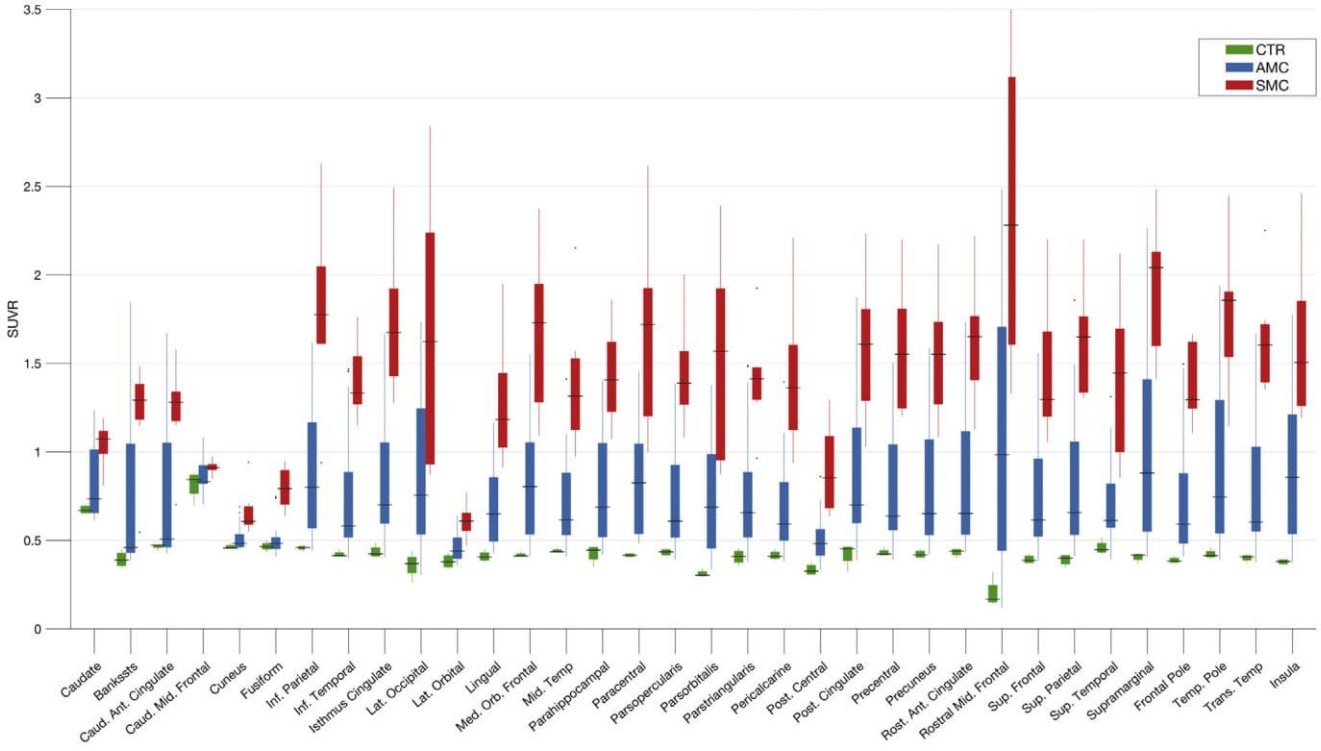

Fig. 3. Mean standardized uptake value ratio (SUVR) within each ROI for controls (CTR), asymptomatic mutation carriers (AMCs), and symptomatic mutation carriers (SMCs). Only areas with significant differences between CTR and AMCs are shown in the figure. Differences between CTR and SMCs are significant for all regions studied.

according to previous findings with other tracers and in other cohorts, we found increased FBB uptake in *PSEN1* MCs in cortical and subcortical areas, found in precuneus, bankssts, supramarginal, superior and inferior parietal and lateral occipital in asymptomatic subjects younger than 2 decades before the predicted symptoms onset, suggesting progressive deposition of fibrillar Aβ. We identified areas with strong correlation between FBB uptake and EYO among which outstands the rostral anterior cingulate, suggesting that this area could be used to track the progressive fibrillar Aβ deposition across the disease. Striatal pathological uptake was observed in 50% of the positive scans, and cerebellar uptake was only in one SMC at visual examination. In addition, we identified areas of increased CTh in AMCs, in accordance with previous studies (Fortea et al., 2010; Sala-Llonch et al., 2015), as the bankssts and the precuneus, which also presented significant amyloid deposition in early AMC stages. Besides not being a longitudinal study, our findings in subjects at different EYO suggest a progressive increase in amyloid deposition when approaching disease onset. In terms of safety, the use of FBB PET was shown to be safe and well tolerated in this population.

Overall, we demonstrate the capability of FBB-PET imaging to depict amyloid deposition changes up to 20 years before the clinical onset of AD. Notably, we described regional patterns similar to those reported in other cohorts with other tracers, besides potential differences in the degree of retention between amyloid binding detected with 18C-PIB and FBB (Villemagne et al., 2012). Studies on the DIAN cohort, both cross-sectionally (Bateman et al., 2012; Benzinger et al., 2013; Oxtoby et al., 2018) and longitudinally (Gordon et al., 2018), reported amyloid deposition in nearly every cortical region 15 years before the estimated age of onset for ADAD MCs, measured with PIB-PET. Also using PIB, another study reported higher striatum uptake in presymptomatic ADAD subjects 17 years before the estimated onset and longitudinal increases in the frontal cortex and in the putamen when subjects approached the age of onset (Rodriguez-Vieitez et al., 2016). In presymptomatic *PSEN1* E280A MCs, using 18F-florbetapir, it has been found that fibrillar Aβ began to accumulate about 16 years before the predicted median age of mild cognitive impairment onset, in the anterior and posterior cingulate, precuneus, parietotemporal and frontal cortex, and basal ganglia (Fleisher et al., 2015). In addition, studies in sporadic AD (Villemagne et al., 2013) have found amyloid PET changes up to 17 years before the stage of mild dementia, suggesting similarities between ADAD and late-onset sporadic AD in the chronology of the progressive amyloid deposition.

In regard to the regional specificity, using PIB, striatal amyloid accumulation was described as a specific feature of ADAD (Klunk et al., 2007). In our sample, using FBB, we find a regional pattern of alterations in ADAD, which was in general characterized by diffuse cortical uptake accompanied by slight striatal alterations. Striatal uptake was frequent but not universal in our subjects. Interestingly, the only SMC who did not show altered FBB uptake in the striatum showed relevant cerebellar uptake. Increased FBB uptake in the caudate appeared in the early presymptomatic phase, whereas hippocampal uptake increase appeared in the last years of the presymptomatic stage, it was significantly higher in stage II-AMCs (less than 15 years before the predicted age of onset), with no differences in younger asymptomatic carriers, compared with noncarriers. In addition, only one of the MCs showed FBB uptake above the cutoff in the hippocampus. Our study did not show relevant amyloid tracer retention in ADAD in the hippocampus, similar to previous studies (Benzinger et al., 2013; Gordon et al., 2018; Rodriguez-Vietez et al., 2016) and in agreement with classical neuropathological studies that revealed the hippocampus only presented mild deposits of amyloid (Braak and Braak, 1991). The rest of subcortical structures studied showed abnormal deposition in SMCs, but not in AMCs.

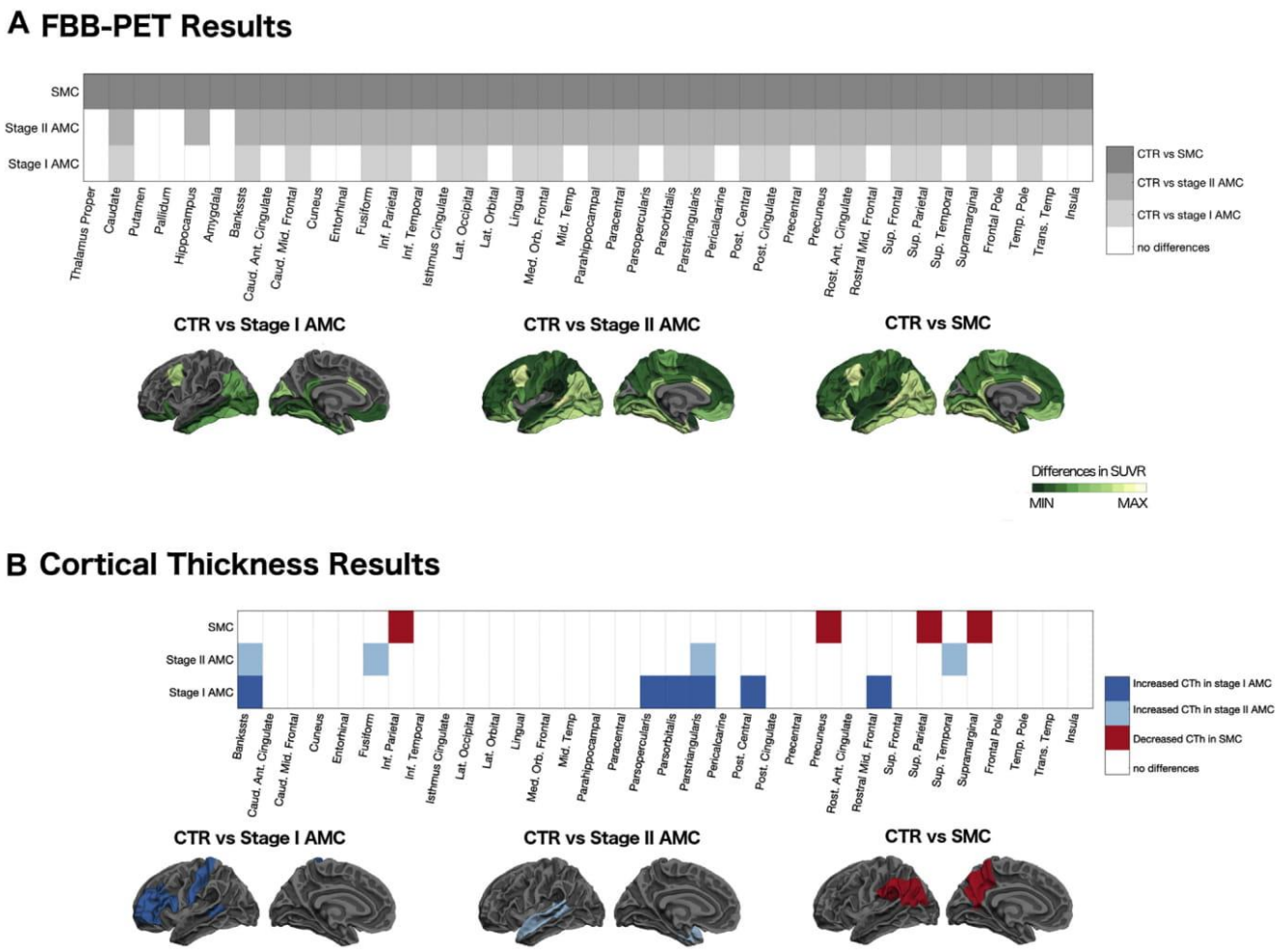

Fig. 4. Summary of the FBB and CTh group comparisons. In (A), areas with significant FBB uptake differences in the comparison with the CTR group ($p < 0.05$ in the Kruskal-Wallis test), as indicated with gray boxes (light gray for stage I-AMCs, medium gray for stage II-AMCs, and dark gray for SMCs). The brain representation shows significant cortical parcellations on a standard surface brain. Green-yellow scale indicates the degree of differences in the SUVR for each region of each group compared with CTR. In (B), areas with significant CTh differences in the comparison with the CTR group ($p < 0.05$ in the Kruskal-Wallis test), as indicated with blue boxes (decreased CTh) and red boxes (increased CTh). The brain representation shows significant cortical parcellations on a standard surface brain. Blue-Red colors indicate the direction of differences for each region of each group compared with CTR, following the same legend as the box grid. See main text for differences surviving multiple comparisons. Abbreviations: AMC, asymptomatic mutation carrier; CTh, cortical thickness; CTR, control; FBB, 18F-florbetaben; SMC, symptomatic mutation carrier; SUVR, standardized uptake value ratio. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

We found strong correlations between FBB uptake and EYO for all MCs in all the regions studied. These were also observed in AMCs, but not in SMCs, suggesting that time does not relevantly affect amyloid accumulation after the symptoms onset. If we exclude the pallidum, which may present unspecific bingeing, considering that CTR also presented high uptake, the anterior rostral cingulate appeared as the region showing strongest correlations in AMCs, and thus, it can be considered as a candidate region to be used to track amyloid deposition (rho = 0.75 in AMCs and 0.83 in MCs). In addition, the precuneus that has been proposed as a candidate region by other authors (Bateman et al., 2012; Benzinger et al., 2013; Gordon et al., 2018), also showed strong correlations in our sample (rho = 0.62 in AMCs and 0.77 in MCs).

In previous studies, we described increased CTh in AMCs compared with CTR, at a mean of 16 years from symptoms onset, in the temporoparietal cortex, precentral and postcentral cortices, and pars triangularis and pars opercularis regions, accompanied by increased caudate volume (Fortea et al., 2010; Sala-Llonch et al., 2015). We then speculated that neuroinflammation and/or accumulation of amyloid species preceding neurodegeneration could account for this phenomenon. Here, we observed that those regions showed increased FBB uptake very early on in the disease course, suggesting pathological fibrillar amyloid deposition in areas with increased thickness (Fortea et al., 2010; Sala-Llonch et al., 2015). In the same line, Rodriguez-Vieitez et al. (2016) described that there is initially high followed by declining astrocytosis in ADAD carriers, at the time of early amyloid deposition, suggesting astrocyte activation is implicated in the early stages of AD pathology (Quiroz et al., 2018).

Notably, in this study, we used a semiautomated method that uses individual anatomical information, PET-MRI registration, and partial volume effect correction (Greve et al., 2016). Even if we have proven that the classification into positive/negative scans is similar to that obtained by visual ratings, automatic quantifications allowed us providing an accurate description of regional alterations, discriminating areas affected differently at each stage of the disease progression.

It is a matter of discussion whether different amyloid tracers bind to identical sites on Aβ fibrils, offering the same ability to detect the regional Aβ burden or not. In this sense, one of the main

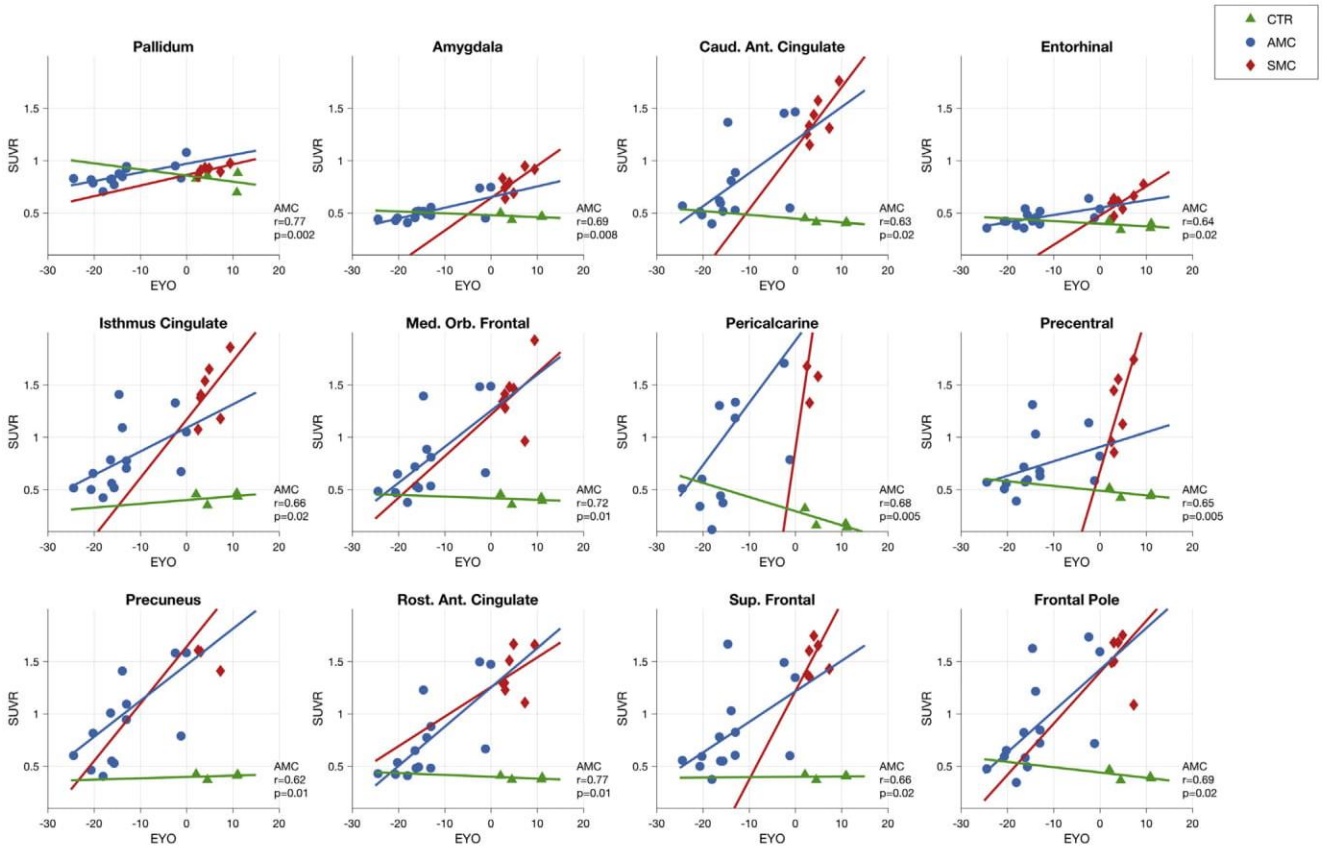

Fig. 5. Correlation between SUVR and EYO for each group. For each region, the result of the Spearman correlation within the AMC group is shown on the bottom-right corner. Only regions with correlation of r > 0.62 are shown and corrected $p < 0.05$. The detailed results of the correlations for all groups and all regions are shown in Supplementary Material. Abbreviations: AMC, asymptomatic mutation carrier; CTR, control; EYO, estimated years to symptoms onset; SMC, symptomatic mutation carrier; SUVR, standardized uptake value ratio.

contributions of our work is the confirmation that FBB is able to detect the amyloid plaques observed in PSEN1 carriers as soon as 2 decades before predicted age of onset. On the other hand, previous studies refer to the precuneus as the preferred area to study the progressive deposition of amyloid using amyloid PET. Here, besides validating the results on the precuneus, we discuss that other areas, as the anterior cingulate, are also good candidates to monitor amyloid uptake.

Overall, even when most of our results are in line with previous studies with other tracers, suggesting that most of the effects described are related to real amyloid deposition and not to an unspecific tracer effect, we should acknowledge some limitations. First, the sample size limits the strength of the results and precluded some of the potential analysis, but on the other hand, it is relevant considering that is a unicentric study and no other data are available with FBB in this population. The second limitation is that this is a cross-sectional study, and even if cross-sectional studies have provided evidences further proved in longitudinal studies, the magnitude of the effect or the temporal pattern of changes may differ with real longitudinal studies. Other limitation is the use of an FBB cutoff positivity that was obtained for sporadic AD. However, the results obtained are congruent with what could be expected according to the literature. Finally, our approach relied on a familial age of symptom onset as a proxy for disease progression, which limits the accuracy of predictions due to the potential imprecision in such estimates (Ryman et al., 2014). Giving that it is currently not possible to predict the exact age of onset for each AMC subject using individual biomarkers, in the future, our results would need to be reanalyzed retrospectively when these AMCs reach the disease onset.

5. Conclusion

In summary, we confirm and expand previous findings of very early, progressive, and region-dependent amyloid tracer uptake in ADAD in an independent unicentric cohort (Barcelona cohort), with a different amyloid tracer, FBB. Our findings would suggest that FBB disclosse a similar spatial pattern and chronology of tracer uptake to that described in previous publications with other amyloid tracers in ADAD.

Disclosure

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neurobiolaging.2019.04.010.

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VII. DISCUSSIÓ

DISCUSSIÓ

Els treballs que conformen la present memòria de tesi doctoral pretenen aportar informació d'aplicabilitat clínica sobre quin és l'impacte clínic de l'ús dels actuals biomarcadors diagnòstics, avaluant quins d'aquests biomarcadors diagnòstics són millor tolerats i augmenten més la confiança diagnòstica, amb l'objectiu final de poder optimitzar el procés diagnòstic a la MAP. Així mateix, el present treball pretén ampliar el coneixement sobre altres biomarcadors que ara per ara s'utilitzen en l'àmbit de recerca per tal d'entendre millor la fisiopatologia de la malaltia i valorar el seu potencial valor diagnòstic i/o pronòstic en la MAP.

El primer treball descrit avalua l'aplicabilitat dels diferents biomarcadors diagnòstics durant el procés diagnòstic de pacients amb deteriorament cognitiu d'inici precoç. Tot i que alguns estudis previs han avaluat la confiança diagnòstica que aportava l'ús d'alguns d'aquests biomarcadors de forma aïllada, aquest és el primer estudi en fer una comparativa entre tots els biomarcadors disponibles en una mateixa cohort de pacients amb MAP (Frisoni et al., 2017; De Wilde et al., 2017). El resultats d'aquest treball suggereixen que a la pràctica clínica diària, l'ús d'aquests biomarcadors tindria un impacte clínic molt rellevant en el procés diagnòstic del deteriorament cognitiu d'inici precoç, ja que comporta un canvi de diagnòstic d'un terç dels pacients. Aquesta dada està en concordança amb publicacions prèvies que demostren percentatges de canvi diagnòstic similars, tot i que majoritàriament s'han avaluat en cohorts de MAT (Rabinovici et al., 2019; De Wilde et al., 2018; Motara et al., 2017; Duits et al., 2015; Mouton-Liger et al., 2014). El fet que hi hagi aquest percentatge de canvi diagnòstic després de les proves, suggereix que en una part important dels pacients que consulten per deteriorament cognitiu a edats primerenques de la vida, l'ús de biomarcadors ens permetria obtenir un diagnòstic més acurat i fiable que el que obtindríem només amb els criteris clínics. Aquests resultats estarien en concordança amb la revisió de cohorts clínico-patològiques (Beach et al., 2012; Balasa et al., 2011). Això, s'explicaria en part, per la major especificitat que ofereixen els biomarcadors respecte als criteris clínics, ja que fenotípicament hi ha un solapament de la MA amb altres patologies, especialment en les formes no amnèsiques. A més, en la nostra cohort, el seu ús també va comportar canvis de tractament en un terç de la mostra, fet que també està en la línia del publicat

prèviament en estudis que avaluen l'impacte de l'ús d'un biomarcador de forma aïllada (De Wilde et al., 2018; Motara et al., 2017). Així doncs, la integració dels biomarcadors en el maneig diagnòstic de MAP també condicionaria en molts casos el seu maneig terapèutic.

La decisió de quin biomarcador utilitzar durant el procés diagnòstic hauria de ser una decisió compartida entre el metge i el pacient i/o la família, per tant a més d'avaluar la utilitat dels biomarcadors des del punt de vista de la confiança diagnòstica que genera en el professional, és important també conèixer l'opinió dels pacients respecte el grau d'invasivitat de les proves diagnòstiques utilitzades (Kunneman et al., 2017). Els resultats mostren que la majoria de pacients no havien tingut cap molèstia, o havien tingut una molèstia lleu, en totes les proves, així com que majoritàriament també les repetirien si fos necessari. Per tant, podem afirmar que en la nostra cohort totes les proves (punció lumbar, PET-FDG, PET-amiloide, RM cerebral) van ser en general ben tolerades. És d'especial rellevància destacar que la punció lumbar, considerada una prova invasiva, va ser molt ben tolerada per la majoria de pacients fet que suggereix que no és necessari limitar-ne el seu ús més enllà de les contraindicacions ja conegudes (tractament anticoagulant, plaquetopènia, alteració de l'hemostàsia), tot i que sempre cal tenir en compte totes les mesures per prevenir-ne els possibles efectes secundaris. Per altra banda, tot i que els resultats de tolerabilitat no van mostrar diferències significatives entre les diferents proves, la RM cerebral va obtenir percentualment el nombre més elevat de molèstia severa que va ser deguda a claustrofòbia, i per tant caldria evitar-la en aquests casos.

A més de l'impacte global dels biomarcadors en el procés diagnòstic i terapèutic del pacient amb MAP i la tolerabilitat de les proves, els resultats del primer treball també suggereix que el valor afegit que aporta cada biomarcador al diagnòstic final del pacient és diferent. En aquest sentit, el PET-amiloide i els biomarcadors a LCR ($A\beta_{42}$ i tau) mostren un increment significatiu en la confiança del clínic tant a l'hora de confirmar el diagnòstic final de MAP, com a l'hora de descartar-lo i establir un diagnòstic de deteriorament cognitiu no neurodegeneratiu, o bé establir el diagnòstic d'una altra malaltia neurodegenerativa com la DFT. El PET-FDG en canvi, no ha demostrat incrementar significativament la confiança diagnòstica en aquests pacients i l'avaluació

visual de l'atròfia d'hipocamp a RM mitjançant escala visual MTA fins i tot l'ha reduïda de forma estadísticament significativa. La superioritat trobada en el nostre treball dels biomarcadors més específics de MA, com el LCR i el PET-amiloide, és concordant amb estudis publicats prèviament que ja suggerien un major increment de la confiança diagnòstica d'aquests biomarcadors envers el PET-FDG o MTA (Bocchetta et al., 2015; Ossenkoppele et al., 2013). El fet que el PET-amiloide i l'anàlisi del LCR proporcionin una mesura directa del canvis anatomopatològics cerebrals des de fases inicials de la malaltia, així com el seu bon rendiment diagnòstic i la bona correlació entre ells, és coherent amb el fet que ambdós biomarcadors incrementin la confiança de forma rellevant i similar entre ells (Weston et al., 2016). Tant el PET-FDG com l'atròfia d'hipocamp a RM cerebral, en canvi, són biomarcadors menys específics i que probablement reflecteixin canvis fisiopatològics que es produeixen en moments més tardans del mateix procés de neurodegeneració. Això pot condicionar que el seu rendiment diagnòstic sigui inferior en fases inicials de la malaltia i en conseqüència, proporcioni una menor confiança diagnòstica (Jack et al., 2013).

Tot i així, l'avaluació visual de l'atròfia d'hipocamp mitjançant l'escala MTA ha sigut àmpliament validada en nombrosos estudis (Ten Kate et al., 2017). Si bé la majoria s'han realitzat en cohorts sense confirmació biològica ni anatomopatològica de la malaltia, l'atròfia d'hipocamp s'ha proposat com a bon predictor de progressió de DCL a MA, així com ha demostrat tenir una bona correlació amb diverses tècniques de quantificació de volum de l'hipocamp (Ten Kate et al., 2017; Ferreira et al., 2015; Varon et al., 2015; Pereira et al., 2014; Duara et al., 2013; Heo et al., 2013; Shen et al., 2011). Tot això, juntament amb el fet que és una eina àmpliament disponible i que a diferència de les tècniques quantitatives, és fàcilment aplicable a la pràctica clínica, va portar a que es proposés com a biomarcador per a l'avaluació de l'atròfia d'hipocamp i que a data d'avui sigui encara la tècnica més utilitzada. Així mateix, es va establir un MTA≥1,5 com a punt de tall considerat com a patològic en pacients per sota dels 75 anys (Van de Pol et al., 2014). El contrast d'aquestes dades amb els resultats obtinguts en el nostre primer treball, junt amb les poques dades disponibles a la literatura de pacients amb MAP, especialment tenint en compte les presentacions atípiques de la malaltia, van motivar

que ens plategéssim avaluar la seva sensibilitat i especificitat diagnòstica en una cohort de pacients amb MAP, utilitzant ambdós mètodes (MTA i volumetria d'hipocamp).

Els resultats obtinguts en aquest segon treball suggereixen que el rendiment diagnòstic de l'avaluació visual de l'atròfia d'hipocamp mitjançant l'escala MTA en pacients amb MAP és subòptim i inferior al rendiment obtingut en pacients amb MAT i inclús, amb altres patologies com la DFT. La sensibilitat de l'atròfia d'hipocamp en pacients amb MAP va ser inferior al 60% per a tots els punts de talls avaluats de MTA (MTA≥1, MTA≥1,5, MTA≥2), sent especialment més baixa en aquells subjectes amb presentacions no amnèsiques. S'ha descrit que les variants no amnèsiques de la MA sovint presenten un patró d'atròfia cerebral predominant en altres àrees cerebrals com ara la parietal, en comptes l'hipocamp (Phillips et al., 2018; Mendez et al., 2017; Rabinovici et al., 2007; Frisoni et al., 2007). Per tant, era esperable que la capacitat diagnòstica de MTA en aquest grup fos inferior que en pacients amb MA amnèsica (Lehman et al., 2012). En canvi, el seu baix rendiment en presentacions amnèsiques és més sorprenent degut a que l'hipocamp és una estructura cerebral directament relacionada amb la memòria i es coneix que fisiopatològicament és la primera estructura en afectar-se en estadis inicials de la malaltia per patologia tau (Sarazin et al., 2010; Braak et al. 1985). El fet que MTA sigui tan poc sensible pel diagnòstic de MAP i que per tant tingui un limitat valor afegit en el procés diagnòstic d'aquests pacients, pot explicar-se llavors, tant per la major freqüència de presentacions no amnèsiques, així com pel fet que al ser pacients més joves l'atròfia cerebral podria ser menys prevalent i/o menys rellevant en les fases inicials de la malatia (Lehman et al., 2012). Així mateix, la capacitat de MTA per discriminar entre la MA i altres patologies (DFT) va ser subòptima ja que les especificitats obtingudes per distingir entre MAT i MAP de DFT van ser inferiors al 40%. Aquest dada posa de rellevància la poca utilitat d'aquesta eina per al diagnòstic diferencial entre MA i la DFT, donat que la mateixa DFT també pot presentar atròfia d'hipocamp (Harper et al., 2016; Hornberger et al., 2012).

El desenvolupament en les últimes dècades de diferents tècniques de mesura volumètrica de l'hipocamp té com a objectiu disposar d'una eina que pugui aportar un benefici en el diagnòstic de la MA respecte la seva avaluació mitjançant l'escala visual (Bosco et al., 2017). En el nostre treball la volumetria va mostrar un rendiment

diagnòstic superior a l'avaluació visual de la MTA per discriminar pacients amb MAP de controls, obtenint el millor punt de tall (6258.75 mm^3) una sensibilitat i especificitat superior al 85%, un resultat similar al descrit a la literatura (Cover et al., 2016; Varon D et al., 2015; Cuingnet et al., 2011; Shen et al., 2011; Ridha et al., 2007; Wahlund LO et al., 2000). Tot i així, malauradament, aquesta tècnica no va aconseguir discriminar la MA d'altres patologies com la DFT en pacients amb deteriorament cognitiu d'inici precoç (Van de Pol et al., 2006). Tenint en compte els criteris descrits prèviament que defineixen que un biomarcador de MA hauria de tenir una sensibilitat i especificitat del 80%, l'atròfia d'hipocamp, ja sigui avaluada visualment o quantitativament, no compliria els criteris diagnòstics per ser-ho en el marc de la MAP, ni tan sols en aquells pacients amb presentacions amnèsiques (The Ronald and Nancy Reagan Research Institute of the Alzheimer's Association and the National Institute on Aging Working Group, 1998).

La poca utilitat diagnòstica de la MTA així com el coneixement de que altres àrees cerebrals més enllà de l'hipocamp poden estar atròfiques a la MAP, ens portar a plantejar un tercer treball. L'objectiu d'aquest va ser valorar si altres escales visuals d'atròfia cerebral descrites a la literatura podrien obtenir un millor rendiment diagnòstic en aquests pacients en fases inicials de la malaltia (DCL i demència lleu). Els resultats van mostrar que les escales amb millor rendiment diagnòstic per discriminar pacients amb MAP de controls eren diferents segons el fenotip clínic. Així, l'escala del cingulat anterior va demostrar ser la millor per detectar pacients amb variant amnèsica de la MA (sensibilitat 66%, especificitat 83%), per sobre del rendiment de la MTA. El cingulat anterior s'ha relacionat amb la flexibilitat de pensament, apatia i desconeixement dels propis dèficits mnèsics (Amazio et al., 2011). Tot i que ha estat poc estudiada la seva relació amb la MA, alguns estudis previs ja havien descrit atròfia en aquest àrea en la MA (Jones et al., 2006). Per altra banda, l'escala d'atròfia posterior, va ser l'escala amb millor rendiment tant per la variant no amnèsica de la MAP (sensibilitat 68%, especificitat 88%) com per la MAG (sensibilitat 86%, especificitat 69%). Aquest resultat està en concordança amb el patró d'atròfia predominant a àrees parietals, descrit prèviament a la literatura per a MAP, especialment en casos no amnèsics (Koedam et al., 2010). Així mateix, els resultats concorden amb els publicats prèviament en una cohort que incloïa subjectes amb MAP amb confirmació anatomopatològica però

en estadis més avançats de la malaltia (Harper et al., 2016). De totes formes, en el nostre treball, tot i que l'escala del cingulat anterior en amnèsics i la d'atròfia posterior en no amnèsics són escales amb millor rendiment diagnòstic, malauradament tampoc compleirien els criteris per a ser considerades biomarcador de MA.

Els rendiments diagnòstics obtinguts tant per l'escala MTA com per la resta d'escales d'atròfia visual, suggereixen una utilitat limitada d'aquestes escales en el diagnòstic de pacients amb deteriorament cognitiu d'inici precoç ja que indiquen que l'absència d'atròfia cerebral en un pacient amb deteriorament cognitiu d'inici precoç, no descarta la possibilitat de patir MAP i si n'hi ha, aquesta és poc específica. Atesos aquest resultats caldria remarcar la necessitat de ser cautes en la interpretació de l'avaluació visual d'atròfia cerebral en la primera valoració d'un pacient amb deteriorament cognitiu d'inici precoç. Així mateix, tenint en compte que hi ha altres biomarcadors neurodegeneratius més específics de la malaltia, com ara P-Tau a LCR, caldria reconsiderar quin valor atorguem a l'avaluació de l'atròfia d'hipocamp dins dels criteris diagnòstics de MA. En aquest sentit, creiem que redefinir l'atròfia d'hipocamp com a criteri o biomarcador de suport en comptes de com a biomarcador diagnòstic, com a mínim en casos de pacients amb MAP, ajudaria a ponderar el seu valor respecte altres biomarcadors que ofereixen un major valor afegit en el procés diagnòstic, especialment quan són aplicats a pacients que inicien la simptomatologia abans dels 65 anys. Aquesta definició estaria al seu torn, més en concordança amb allò descrit recentment en els nous criteris de recerca a la MA (Jack et al., 2018).

Els darrers treballs de la present tesis doctoral han sigut dirigits a l'estudi de biomarcadors, tant bioquímics en LCR com de neuroimatge, que podrien tenir un paper rellevant en la fisiopatologia de la malaltia. La majoria s'han desenvolupat en els últims anys i l'ús d'alguns d'ells encara està limitat a l'àmbit de la recerca.

En el quart treball presentat vam avaluar el perfil de biomarcadors a LCR i la seva relació amb els canvis estructurals cerebrals (RM cerebral) característics de pacients amb MA. Vam incloure tant biomarcadors típics ($A\beta_{42,}$ T-Tau i P-Tau), com nous biomarcadors (Ng i NfL) així com d'altres que, tot i que coneguts, el seu paper dins la MA ha estat menys estudiat (14-3-3) (Mattson et al., 2017). Tot i que la relació entre les

característiques en neuroimatge de la MA i els seus biomarcadors típics de LCR han estat estudiats tant en fases clíniques com preclíniques de la malaltia, és encara incert quina relació o influència poden tenir els nous biomarcadors sobre els patrons d'atròfia a la MAP (Pegueroles et al., 2017; Idland et al., 2017; Fortea et al., 2014; Tosun et al., 2011).

Els resultats descriptius dels biomarcadors a LCR van mostrar que a més del perfil clàssic de la malaltia, consistent en la disminució dels nivells de $A\beta_{42}$ i elevació de T-Tau i P-Tau, la concentració a LCR de la resta de biomarcadors (Ng, Nfl i 14-3-3) també estava elevada comparat amb controls. Aquests resultats són concordants amb el descrit prèviament (Blennow et al., 2018; Portelius et al., 2018; Rohrer et al., 2016; Burkhard et al., 2001).

La signatura típica de la MAP a la nostra cohort, definida pel patró de canvis estructurals a la RM, va consistir en la disminució generalitzada de gruix cortical (substància gris), la pèrdua generalitzada d'integritat de la substància blanca així com pèrdua de volum d'estructures subcorticals (hipocamp), en la línia del publicat a la literatura (Möller et al. 2015, Rabinovici et al. 2007). El biomarcador que va destacar com a principal predictor de l'atròfia cerebral a la MAP, tant a substància gris com a substància blanca, va ser els nivells de la proteïna $A\beta_{42.}$ Tot i així el seu efecte sobre la pèrdua de gruix cortical es va veure complementat per la 14-3-3, que es va erigir com a segon biomarcador en importància relativa. Pel que fa al dany a substància blanca, a més de la $A\beta_{42}$, la pèrdua de volum a aquest nivell també es va relacionar amb T-Tau i l'edat.

El predomini de la influència dels biomarcadors $A\beta_{42}$ i T-Tau en les alteracions estructurals cerebrals típiques de la MA són esperables degut a que conformen el segell distintiu dels principals canvis fisiopatològics de la MA. L'efecte d'aquest biomarcadors sobre el patró d'atròfia cerebral està en consonància amb allò descrit, fet que suggereix que aquests biomarcadors diagnòstics, són també uns bons descriptors de les alteracions estructurals que tenen lloc a la MA (Blennow et al., 2018; Pereira et al., 2017; Ossenkoppele et al., 2015; Tosun et al., 2011). De totes formes, els resultats d'aquest treball posen també en rellevància la participació d'altres proteïnes més enllà dels biomarcadors típics de la malaltia, i per tant, destaca la complexitat del procés

fisiopatològic de la MAP. Així, la proteïna 14-3-3, ha mostrat la seva contribució als canvis observats a substància gris. Aquest és un fet plausible ja que tot i que inespecífica, la 14-3-3 ha demostrat tenir un paper en els procés neurodegeneratiu de la MA (Burkhard et al., 2001; McFerrin et al., 2017). Cal destacar per altra banda l'absència de NfL i Ng com a factors influents en el patró d'atròfia cerebral a MAP. En el cas de NfL, pot explicar-se pel fet que tot i que la seva presència en MA ha estat ben demostrada, aquest és un biomarcador amb més pes a altres patologies com la DFT, on ha demostrat tenir concentracions més elevades així com implicacions pronòstiques de la malaltia (Steinacker et al., 2018; Rohrer et al., 2016; Pijnenburg et al., 2007). Per altra banda, la rellevant correlació estadística obtinguda entre Nfl i T-Tau pot haver comportat la neutralització de l'efecte de NfL en l'estudi multivariant. A la nostra cohort, en concordança amb el prèviament publicat, es va observar una major concentració de NfL a DFT que a MA, així com també va ser el biomarcador que més va contribuir al patró d'atròfia cerebral a la DFT. La Ng en canvi, encara que s'ha definit com a biomarcador específic de MA, hi ha estudis recents que han suggerit que no aporta un valor afegit respecte als biomarcadors típics de la MA i han posat en dubte la seva utilitat diagnòstica (Lista et al., 2017).

Com hem vist al llarg de la discussió, els biomarcadors de dipòsit de proteïna amiloide tenen un paper especialment important a nivell diagnòstic de la MAP i també a l'hora d'entendre la fisiopatologia de MAP. Això és degut a que la seva detecció tradueix un dels principals canvis anatomopatològics coneguts que tenen lloc a la MA. El PET-amiloide és una de les eines que ens permet la seva detecció, i aplicat a cohorts de MAG ens ofereix la possibilitat d'estudiar la trajectòria d'aquest biomarcador al llarg de tota la malaltia, des de fases preclíniques a clíniques de la malaltia (Bateman et al., 2011).

Per aquest motiu, en el cinquè treball, vam voler avaluar la seguretat i eficàcia del PET-FBB per detectar patrons de dipòsit de MA en una cohort de MAG amb mutació en *PSEN1*, incloent tant subjectes asimptomàtics com simptomàtics. Els resultats van mostrar que el PET-FBB és una tècnica segura donat que no hi va haver cap efecte advers derivat de la realització de la prova. Així mateix, la seva lectura tant visual com semi-quantitativa va permetre classificar de forma dicotòmica el resultat de la prova. Tot i així la lectura semi-automàtica va permetre aprofundir en l'estudi detallat del grau

d'afectació de cada regió cerebral. Els resultats van mostrar un increment de la captació de florbetaben en els portadors de la mutació de *PSEN1*, tant a nivell cortical com subcortical. Aquesta captació era present ja en el portadors asimptomàtics, fins a dues dècades abans de l'inici dels símptomes. Aquesta troballa dona suport a la hipòtesi que els primers canvis de la malaltia tenen lloc en fases preclíniques precoces i coincideix amb el descrit prèviament amb els traçadors *11C-Pittsburgh compound B* (Gordon et al., 2018; Benzinguer et al., 2013; Bateman et al., 2012) i florbetapir (Fleisher et al., 2012). Pel que fa a patrons de captació, el més freqüent a la nostra mostra va ser la captació cortical difusa amb captació estriatal associada. La captació estriatal s'havia proposat prèviament com una característica específica a la MAG donat que no és una troballa freqüent a la MA esporàdica (Benzinguer 2013). Tot i que aquesta captació s'havia demostrat prèviament utilitzant el traçador *11C-Pittsburgh compound B,* no s'havia confirmat amb un altre traçador como el florbetaben. En la nostra cohort, la captació estriatal va ser freqüent però no universal de manera similar a altres cohorts estudiades amb altres traçadors (Klunk et al., 2017). Per altra banda, també es va detectar una captació precoç al nucli caudat en fases presimptomàtiques de la malaltia, i en canvi l'hipocamp no va mostrar una afectació rellevant, resultats que estan en concordança amb estudis previs (Gordon et al., 2018; Betzinguer et al., 2013; Braak and Braak, 1991). Es van identificar també diverses regions que mostraven una forta correlació entre la captació de FBB i la edat estimada d'inici dels símptomes, sent especialment rellevant en la regió del cingulat anterior. Així mateix, el precuneus també va mostrar una correlació forta, de forma similar al descrit prèviament (Gordon et al., 2018; Benzinguer et al., 2013; Bateman et al., 2012). Això suggereix que, més enllà del precuneus, el cingulat anterior també podria ser una regió útil per la monitorització del dipòsit de proteïna amiloide. Per últim, es va observar un increment del gruix cortical regional a RM en subjectes portadors asimptomàtics en àrees com el còrtex temporo-parietal, suggerint que aquests canvis podrien estar en relació amb l'existència d'un dipòsit inicial de proteïna amiloide en fases precoces de la malaltia, tal i com s'havia descrit prèviament (Sala-Llonch et al., 2015; Fortea et al., 2010).

VIII. CONCLUSIONS

CONCLUSIONS

1. L'ús dels biomarcadors diagnòstics tenen un impacte clínic rellevant en el procés diagnòstic de la MAP
    a. L'ús de biomarcadors en el diagnòstic de la MAP comporta un canvi de diagnòstic i de tractament en un terç dels casos.
    b. Els biomarcadors $A\beta_{42,}$ T-Tau i P-Tau a LCR i el PET-amiloïd són els biomarcadors més útils en el procés diagnòstic de MAP donat que incrementen de forma significativa la certesa diagnòstica. En canvi, el PET-FDG i l'atròfia d'hipocamp a RM no han demostrat proporcionar un valor diagnòstic afegit.
    c. Les proves que es realitzen per obtenir els biomarcadors diagnòstics (punció lumbar, PET-FDG, PET-amiloide, RM cerebral) són segures i ben tolerades per part dels pacients.

2. L'avaluació visual de l'atròfia d'hipocamp (escala MTA) té una utilitat limitada en el diagnòstic de la MAP. La tècnica semiautomàtica (plataforma NeuGRID) per mesurar la volumetria d'hipocamp millora el rendiment diagnòstic d'aquest biomarcador al discriminar de MAP de controls, però no al discriminar-la d'altres neurodegeneratives (DFT). Cap de les dues tènciques compleixen criteris per ser considerats biomarcadors diagnòstic de MA en aquests pacients.

3. En la variant amnèsica de la MAP, l'escala que obté un millor rendiment diagnòstic és l'escala del cingulat anterior, a diferència de la variant no amnèsica en què l'escala amb millor rendiment és l'escala posterior. Tot i així, totes les escales visuals d'atròfia cerebral utilitzades a la pràctica clínica (orbito-frontal, cingular anterior, fronto-insular, temporal anterior, posterior) tenen un rendiment diagnòstic subòptim i cap d'elles compleix els criteris per ser considerades biomarcador diagnòstic de MAP.

4. En els anàlisis de regressió múltiple els marcadors que expliquen millor els valors de gruix cortical a la signatura típica de MAP són $A\beta_{42}$ i 14-3-3, mentre que

$A\beta_{42}$ i T-Tau són els que millor expliquen els valors d'integritat de subtància blanca. En canvi, NfL i Ng no contribueix al model.

5. L'ús de PET-FBB és segur i permet identificar una acumulació progressiva de proteïna amiloide a la MAG amb un patró espaial característic que comença fins a dues dècades abans de l'inici dels símptomes. El patró visual més freqüent és la captació generatizada cortical associada a captació estriatal. La captació de florbetaben al cingulat anterior rostral podria ser una regió adequada per monitorizar el dipòsit progressiu de Aβ en portadors de la mutació a *PSEN1*.

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