Factores neuropsicológicos y genéticos asociados a la demencia incipiente desde el Deterioro Cognitivo Leve. Correlación fenotipo-genotipo

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Que en la tesis titulada *"**Factores neuropsicológicos y genéticos asociados a la demencia incipiente desde el Deterioro Cognitivo Leve. Correlación fenotipo-genotipo"*** presentada por Ana Espinosa Cardiel, las siguientes cuatro publicaciones no han sido usadas ni se usarán en futuras tesis:

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En revisión.

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Con el visto bueno de:

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A mi familia.

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1. GLOSARIO DE ABREVIATURAS

ABCA7:	*ATP-binding cassette subfamily A member 7.*
ADGC:	*Alzheimer's Disease Genetics Consortium.*
ADNI:	*Alzheimer's Disease Neuroimaging Initiative.*
AP2A2:	*Adaptor Related Protein Complex 2 Alpha 2 Subunit.*
APOE:	Apolipoproteína E.
APP:	*Amyloid Precursor Protein.*
APP:	Afasia Progresiva Primaria.
ATP5H/KCTD2:	*Adenosine triphosphate (ATP) synthase, H+ transporting, mitochondrial F0/Potassium channel tetramerization domain-containing protein 2.*
AVD:	Actividades de la Vida Diaria.
βA:	Beta amiloide.
BIN1:	*Bridging integrator 1.*
CASS4:	*Cas scaffolding protein family member 4.*
CD2AP:	*CD2-associated protein.*
CD33:	Molécula CD33.
CDR:	*Clinical Dementia Rating.*
CELF1:	*Elav-like family member 1.*
CHARGE:	*Cohorts for Heart and Aging in Genomic Epidemiology.*
CLU/APOJ:	*Clusterin, CLU*/ apolipoprotein J.
Cognitive Composites:	*Composites* Cognitivos.
CR1:	*Complement receptor type 1.*
DCL:	Deterioro Cognitivo Leve.
DCL-a:	Deterioro Cognitivo Leve-amnésico.
DCL-a-md:	Deterioro Cognitivo Leve amnésico múltiple dominio.

GLOSARIO DE ABREVIATURAS

DCL-a-Pr:	Deterioro Cognitivo Leve amnésico probable.
DCL-a-Ps:	Deterioro Cognitivo Leve amnésico posible.
DCL-a-ud:	Deterioro Cognitivo Leve amnésico único dominio.
DCL-na:	Deterioro Cognitivo Leve no amnésico.
DCL-na-md:	Deterioro Cognitivo Leve no amnésico múltiple dominio.
DCL-na-Pr:	Deterioro Cognitivo Leve no amnésico probable.
DCL-na-Ps:	Deterioro Cognitivo Leve no amnésico posible.
DCL-na-ud:	Deterioro Cognitivo Leve no amnésico único dominio.
DCLewy:	Demencia por Cuerpos de Lewy.
DFT:	Demencia Frontotemporal.
DFTvc:	Demencia Frontotemporal variante de conducta.
DLFT:	Degeneración Lobar Frontotemporal.
DLFT-FUS:	Degeneración Lobar Frontotemporal asociada a la proteína de fusión en sarcoma.
DM:	Demencia Mixta (EA con enfermedad cerebrovascular asociada).
DS:	Demencia Semántica.
DV:	Demencia Vascular.
EA:	Enfermedad de Alzheimer.
EADI:	*European Alzheimer's Disease Initiative.*
EOAD:	*Early-onset AD.*
EPHA1:	*EPH receptor A1.*
EXOC3L2:	*Exocyst complex component 3-like 2.*
FERMT2:	*Fermitin family member 2.*
FI:	Factor de impacto.

GDS:	*Global Deterioration Scale.*
GERARD:	*Genetic and Environmental Risk in Alzheimer's Disease.*
GWAS:	Genome-wide association study.
HLA-DRB5-HLA-DRB1:	*Major histocompatibility complex, class II, DR beta 5.*
HS3ST1:	*Heparan Sulfate-Glucosamine 3-Sulfotransferase 1.*
IGAP:	*International Genomics Alzheimer's Project.*
INPP5D:	*Inositol polyphosphate-5-phosphatase D.*
LCR:	Líquido céfalo raquídeo.
LOAD:	*Late-onset AD.*
***MEF2C*:**	*Myocyte enhancer factor 2C.*
MIS:	*Memory Impariment Screen.*
MMSE:	*Mini-Mental State-Examination.*
MS4A:	*Membrane-spanning 4-domains subfamily A.*
NBACE:	Neuropsychological Battery of Fundació ACE.
NME8 el NME/NM23:	*Family member 8.*
PET-FDG:	Tomografía por Emisión Positrones Flúor-18 Flúordeoxiglucosa.
PGS:	*Combinations of SNPs in polygenic scores.*
PIC:	*Polymorphism information content.*
PICALM:	*Phosphatidylinositol binding clathrin assembly protein.*
PSEN1:	Presenilina-1.
PSEN2:	Presenilina-2.
PTK2B:	*PTK2B protein tyrosine kinase 2 beta.*
RM:	Resonancia Magnética.
ROCF:	*Rey-Osterrieth Complex Figure.*

GLOSARIO DE ABREVIATURAS

SLC24A4-RIN3:	*Solute Carrier Family 24 Sodium/Potassium/Calcium Exchanger), Member 4-Ras and Rab Interactor 3.*
SNP:	*Single Nucleotide Polymorphism.*
SORL1:	*Sortilin related receptor 1.*
SPECT:	Tomografía computarizada por emisión de fotón único.
SUVR:	Ratio del Valor de Captación Estandarizado.
T@M:	Test de Alteración de Memoria.
T7M:	Test de los 7-Minutos.
TC:	Tomografía computarizada.
TMT:	*Trail Making Test.*
TREM2:	*Triggering receptor expressed on myeloid cells 2.*
ZCWPW1:	*Zinc finger CW-type and PWWP domain containing.*

2. RESUMEN

ANTECEDENTES: Los estudios sobre el Deterioro Cognitivo Leve (DCL) se centran en buscar predictores de conversión a demencia, principalmente Enfermedad de Alzheimer (EA). No obstante, las clasificaciones de DCL no han integrado los nuevos datos genéticos en la EA. OBJETIVOS: 1. Determinar el riesgo de conversión a demencia y detección de los principales factores de riesgo implicados, en una muestra de 550 pacientes con DCL. 2. Evaluación del efecto de 23 marcadores genéticos sobre la conversión a EA en 1.250 sujetos con DCL. 3. Identificación de modelos predictivos univariantes y multivariantes de conversión a EA mediante técnicas estadísticas y estudios de supervivencia. 4. Replicación de los marcadores genéticos significativos en series adicionales de DCL. 5. Identificación de elementos redundantes entre los marcadores genotipados y priorización racional de los más predictivos. 6. Realizar un meta-análisis de los resultados más relevantes. 7. Analizar la asociación entre patrones neuropsicológicos y marcadores genéticos. MÉTODO: Estudio retrospectivo con 550 sujetos mayores de 60 años diagnosticados de DCL en Fundació ACE (ACE), según criterios de Petersen et al.[138,142] y López et al.[102,103] clasificados en cuatro subtipos: amnésico probable (DCL-a-Pr) (n=115), no-amnésico probable (DCL-na-Pr) (n=37), amnésico posible (DCL-a-Ps) (n=234) y no-amnésico posible (DCL-na-Ps) (n=164) con afectación en un único o múltiples dominios. Se realizaron Análisis de la Varianza (ANOVA), Chi-Cuadrado y análisis de supervivencia (estimador Kaplan Meier; Regresiones de Cox) para valorar conversión. Estudios prospectivos: 1) con biomarcadores de neuroimagen: muestra de DCL-a-Pr (n=20) de ACE con Resonancia Magnética (RM) estructural, tomografía por emisión de positrones (PET) con ^{18}F-fluorodesoxiglucosa (PET-FDG) y PET con Compuesto B de Pittsburgh (N-Methyl-[11C]2-(4'-methylaminophenyl) 6-hydroxy-benzotriazol (PET-PIB). La construcción de cinco *Composites* Cognitivos, las medidas del volumen hipocampal ajustado (VHa) y

del ratio del valor de captación estandarizado (SUVR) del PET-FDG se replicaron en la base de datos parental AB255 (n=133 DCL-a-Pr de almacenamiento). Se correlacionaron, ajustando por edad, género y escolaridad, los cinco *Composites* Cognitivos y el VHa, el SUVR PET-FDG y el SUVR PET-PIB. Replicación con la base de datos "Alzheimer's Disease Neuroimaging Initiative" (ADNI). 2) Correlación fenotipo-genotipo: muestra de DCL (n=1.250), divididos en cuatro subtipos: DCL-a-Pr (n= 262), DCL-na-Pr (n= 76), DCL-a-Ps (n= 549) y DCL-na-Ps (n= 358) con afectación en un único o múltiples dominios, que cumplían los criterios de inclusión del estudio retrospectivo, con evaluación basal clínica y seguimiento anual para verificar su conversión a EA. Genotipado de ADN germinal mediante tecnología Sequenom. Se realizaron análisis de supervivencia y de regresión lineal mediante PLINK, replicación en series independientes y meta-análisis. Análisis de asociación basal entre endofenotipos neuropsicológicos y marcadores genéticos. RESULTADOS: El fenotipo clínico DCL-a-Pr (de almacenamiento) obtuvo 8,5 veces más riesgo de conversión a demencia, principalmente EA, que el DCL-na-Ps, con el menor riesgo y tasa de conversión a demencia. Los endofenotipos neuropsicológicos que mejor predijeron conversión a demencia para la muestra de DCL fueron la Orientación a la Realidad, Recuerdo Diferido de la Lista Palabras WMS-III y Relojes de Luria. El *Composite* Cognitivo Recuerdo Diferido, correlacionó con los tres biomarcadores de neuroimagen asociados a la EA prodrómica. Se identificó el *locus CLU* como factor genético independiente de *APOE*-ε4 asociado a fenoconversión de DCL a EA y se halló un efecto protector del *locus HS3ST1* (rs6448799) asociado a Dígitos Inversos y un efecto de riesgo del *locus AP2A2* (rs10751667) asociado a Repetición verbal, en el fenotipo DCL-a-Pr.

3. INTRODUCCIÓN

3.1 El Deterioro Cognitivo Leve (DCL)

El Deterioro Cognitivo Leve (DCL) es una entidad nosológica que pretende describir el declive de las funciones cognitivas de un individuo, con respecto de su nivel previo y que en general no afecta a la realización de las actividades de la vida diaria (AVD). [139] En términos generales, el DCL suele definirse por la presencia de quejas cognitivas subjetivas y/u objetivas que no son suficientemente severas como para cumplir los criterios de demencia. Sin embargo, el diagnóstico de DCL es un constructo neuropsicológico formulado con fines predictivos, que ha ido evolucionando a lo largo de los años con la finalidad de identificar aquellos factores intrínsecos que aumenten el riesgo de conversión a demencia, particularmente a Enfermedad de Alzheimer (EA).[140]

El deterioro de la memoria episódica es el síntoma más importante para ambos síndromes, la demencia tipo Alzheimer y el DCL, que suele constituir la manifestación clínica más temprana de la EA.[59,73,120,121,142] De hecho, a lo largo de la historia, la necesidad de identificar a aquellos pacientes sin demencia, pero con un riesgo incrementado de desarrollarla, ha derivado en diferentes conceptos tales como: “pérdida de memoria benigna o maligna asociada al envejecimiento”,[97] el “deterioro de memoria asociado a la edad”,[43] o bien, “el deterioro de memoria consistente con la edad” versus “el olvido de la senectud o de la vejez”.[20] Sin embargo, el término DCL fue acuñado por primera vez, en la década de los 80 por un grupo de investigadores del Centro de Investigación de Envejecimiento y Demencia de la Universidad de Nueva York,[63] para describir a aquellos pacientes con dificultades cognitivas detectables y cuantificables, pero sin criterios de demencia. Lo equipararon a

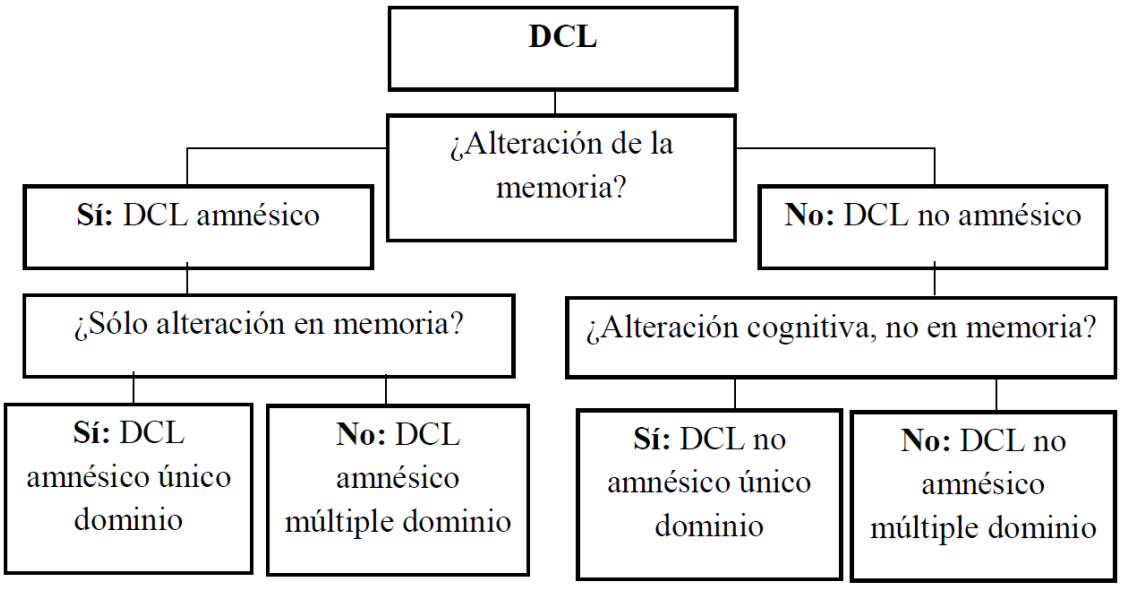
un estadio 3 de la *Global Deterioration Scale* (GDS).[154] Así, se considera como una etapa de transición entre la normalidad y la demencia.[138,188]

La escala GDS clasifica la EA en siete estadios, de los cuales los cuatro primeros abarcan la transición de la normalidad cognitiva sin quejas cognitivas (GDS 1), con quejas cognitivas (GDS 2), el deterioro cognitivo "sutil", que puede repercutir en las actividades complejas de la vida laboral y social (GDS 3), hasta el estadio de demencia leve (GDS 4). Paralelamente, también en la década de los años 80, se desarrolló la escala *Clinical Dementia Rating* (CDR)[119] para cuantificar la gravedad de la demencia. Así, permite diferenciar a los sujetos cognitivamente sanos (CDR 0) de aquellos con DCL (CDR 0,5) y con demencia leve (CDR 1), moderada (CDR 2) o severa (CDR 3). Aunque las escalas de clasificación GDS y CDR no deberían utilizarse como términos intercambiables, ya que no son estrictamente equivalentes, son las más ampliamente utilizadas en el ámbito clínico y de investigación para poder categorizar a los sujetos con DCL. Estos se situarían en la fase intermedia entre la normalidad y la demencia y correspondería, así, con un estadio GDS 3 y CDR 0,5.

3.1.1. Subtipos de DCL

El DCL es una entidad clínica heterogénea. Según la clasificación de Petersen et al. (2004),[138] el DCL se puede dividir en cuatro subgrupos en función del rendimiento en memoria y el número de funciones cognitivas alteradas: amnésico único (DCL-a-ud) o múltiple dominio (DCL-a-md) y no amnésico único (DCL-na-ud) o múltiple dominio (DCL-na-md) (Figura 1).

Figura 1. Esquema de los subtipos de DCL (Petersen et al.)[138]



De los subtipos de DCL que cursan con pérdida de memoria, el amnésico (DCL-a) de Petersen et al. (1999)[142] ha sido el mejor definido y el más utilizado en las últimas décadas, tanto en estudios de investigación como en ensayos clínicos. Los criterios de DCL-a de la Clínica Mayo incluyen: 1) Pérdida subjetiva de memoria, referida por el paciente o por un informador fiable, 2) Cognición global preservada, 3) Normalidad en las actividades de la vida diaria (AVD), 4) Alteración de la memoria para la edad y nivel educativo del sujeto objetivada mediante los test cognitivos y 5) Ausencia de criterios diagnósticos de demencia.[142,143] Aunque estos criterios requieren el uso de las pruebas neuropsicológicas para objetivar la alteración de la memoria, en el subtipo DCL-a-ud, no especifican qué pruebas de memoria deben administrarse, ni el criterio psicométrico para considerar que se encuentra alterada. Sin embargo, la mayoría de autores acepta que la memoria episódica es la que debe ser evaluada, [59,73,120,121,142] y que un rendimiento por debajo de 1,5 desviaciones estándar (DE)

en un dominio, estaría alterado dado que la cohorte de sujetos con DCL de la Clínica Mayo obtuvo un rendimiento en memoria por debajo de 1,5 DE respecto a sus coetáneos. Así, las puntuaciones en las pruebas cognitivas para los individuos con DCL son típicamente 1-1,5 DE por debajo de la media para su edad y nivel de escolaridad en la equiparación con sus iguales en los datos normativos culturalmente apropiados (p.ej. para el/los dominio/s alterado/s), cuando están disponibles. Sin embargo, estos rangos se consideran pautas y no puntos de corte.[4]

Aunque la primera clasificación del DCL se basó en el deterioro únicamente de la memoria (DCL-a-ud), posteriormente se expandió a otros dominios cognitivos, tales como el lenguaje, las funciones ejecutivas o visuoespaciales. Por lo tanto, el subtipo DCL-a-md incluye la alteración de la memoria y de otro/s dominio/s cognitivo/s tales como el lenguaje, praxias y/o la capacidad visuoespacial, visuoperceptiva y ejecutiva. Por el contrario, el subtipo DCL-na-ud engloba a aquellos sujetos que muestran alteración de un área cognitiva diferente de la memoria. Asimismo, los sujetos con DCL DCL-na-md muestran alteración en más de un área cognitiva, con preservación de la memoria.

Cada subtipo de DCL había sido inicialmente relacionado con diferentes demencias de la siguiente manera: DCL-a-ud y DCL-a-md de etiología degenerativa con la EA; DCL-a-md de etiología vascular con la Demencia Vascular (DV); DCL-na-md con la Demencia por Cuerpos de Lewy (DCLewy); DCL-na-md con la DV y DCL-na-ud con la Demencia Frontotemporal (DFT) o DCLewy.[126, 138, 188] Aunque inicialmente

algunos estudios sugerían que, a diferencia del DCL-a, los sujetos con DCL-na muestran una mayor tendencia de conversión a demencias de tipo no Alzheimer,[138, 188] actualmente existe un consenso de que todos los subtipos de DCL convierten principalmente a EA.[52,61,91,164,171]

La tasa de conversión anual del DCL-a a EA oscila entre un 10-25%,[6,63,120,142] o hasta un 30-40%, [14,68,165,169] dependiendo del tipo de estudio (p.ej.: epidemiológico o clínico). Existen numerosas publicaciones sobre qué tipos de DCL representan un mayor riesgo de conversión a demencia, pero sus resultados son contradictorios. Estudios recientes sugieren que el riesgo de EA incrementa cuando otros dominios, además de la memoria, se hallan alterados, porque se encontrarían en un estadio más avanzado de enfermedad neurodegenerativa.[10,126,166,174] Por el contrario, otros estudios han encontrado tasas más bajas de conversión a EA en los DCL-a-md y DCL-na, que en aquellos con DCL-a-ud, lo que sugiere que otras variables neuropsicológicas que no son la memoria no serían útiles para predecir la progresión de la EA. [17,106,169] Así pues, el riesgo de conversión a demencia depende en gran medida de la definición aplicada del DCL y sus criterios neuropsicológicos específicos.

Una clasificación complementaria al DCL amnésico único y múltiple dominio consiste en la presentada por el equipo del *Cardiovascular Health Study (CHS).* [102,103] Los sujetos se clasifican como DCL posible si existe algún factor que justifique sus déficits cognitivos y como DCL probable cuando no se hallaba ninguna comorbilidad. Así pues,

los sujetos con DCL posible pueden padecer enfermedades psiquiátricas (p.ej.: depresión, trastorno bipolar), enfermedades sistémicas neurológicas (p.ej.: accidentes cerebrovasculares, traumatismos craneoencefálicos) o bien no bien no se dispone de suficiente información para refinar su diagnóstico (p.ej.: ausencia de pruebas de neuroimagen, ausencia de un informador fiable).

Mientras que los sujetos con diagnóstico de DCL probable identificaban a aquellos individuos en las primeras etapas de una enfermedad neurodegenerativa, sobre todo debida a la EA, el diagnóstico de DCL posible suele comprender un grupo más heterogéneo de individuos. Los criterios clínicos para el diagnóstico del DCL probable según criterios del CHS[102] son los siguientes: 1) Quejas de memoria referidas por los familiares o el personal clínico, 2) Alteración objetivada (-1,5 DE) mediante pruebas de memoria visual o verbal, 3) La alteración de la memoria representa una afectación respecto al nivel previo, 4) Rendimiento preservado en las otras funciones cognitivas y, 5) Ausencia de otras enfermedades que puedan explicar la afectación cognitiva. A pesar de ello, el DCL posible tenía sólo una tasa ligeramente más baja de conversión a demencia que el grupo de DCL probable, lo cual sugiere que, incluso con condiciones de comorbilidad, existe una alta probabilidad de encontrar una enfermedad neurodegenerativa subyacente.[103] De hecho, estudios sobre las fases incipientes de la DV publican porcentajes de conversión más bajos a demencia que los estudios sobre EA.[67,91,116] Dado que tanto el riesgo de desarrollar la EA como el de sufrir enfermedades cerebrovasculares aumenta con la edad,[122,192] la causa más común de demencia es la combinación de ambas, es decir, la Demencia Mixta (DM).[101,170].

3.1.2. EA prodrómica/predemencia o DCL debido a EA

El concepto de DCL, considerado como el estadio prodrómico de la EA o como la EA temprana, se aplica para describir a aquellos pacientes con déficits de memoria (u otra función cognitiva), que a pesar de no cumplir criterios de demencia tienen alto riesgo de conversión a EA. De este modo, el constructo DCL utiliza criterios relativamente específicos de diagnóstico y aquellos sujetos que parecen presentarlos tienen un riesgo incrementado de desarrollar la EA. [120,138,142,180] Recientemente, dada la creciente evidencia de que el DCL representa un constructo independiente del envejecimiento normal y la EA probable, se ha propuesto la necesidad de incluirlo como una nueva categoría diagnóstica en la quinta edición del Manual Diagnóstico y Estadístico de Trastornos Mentales (DSM-V);[11] basándose en datos clínicos, de seguimiento, epidemiológicos, de neuroimagen y neurofisiológicos.[137]

Si pudiésemos identificar las diferentes enfermedades que producen demencia desde su fase prodrómica/predemencia (es decir, en la fase DCL) o incluso preclínica (quejas cognitivas subjetivas), detectando a aquellos sujetos que evolucionarán hacia demencia (EA u otra), el término DCL dejaría de tener sentido, puesto que se podrían diagnosticar las enfermedades por su nombre en una fase mucho más precoz de lo que los criterios diagnósticos actuales convencionales permiten.[51,52] Así pues, la planificación del cuidado de los pacientes con DCL requiere detectar precozmente a aquellos sujetos con mayor riesgo de conversión a demencia. Su intervención, combinando los tratamientos farmacológicos y no farmacológicos, podría permitir disminuir en un futuro su prevalencia.[176]

No obstante, la detección de los primeros cambios cognitivos en la EA, incluyendo su fase prodrómica o DCL, es un tema complejo. En las últimas décadas la mayor parte de los estudios sobre el DCL se han centrado en la detección de los principales factores predictores de conversión a demencia, principalmente la demencia tipo Alzheimer.[184,185] Recientemente, el Grupo de Trabajo Internacional para la Investigación de Nuevos Criterios para el diagnóstico de la EA introdujo la definición de la EA prodrómica para describir una fase sintomática de la enfermedad o su etapa predemencia.[51]

Dada la importancia de la detección precoz de la demencia, la búsqueda de biomarcadores en sus fases prodrómicas se ha centrado en diferentes métodos, tales como el genotipado de la apolipoproteína E (*APOE*),[33,55] técnicas de neuroimagen estructurales y funcionales,[38,85,94] los test bioquímicos de flujo sanguíneo y cerebroespinal,[110] o pruebas neuropsicológicas. [7,118,126,168,177]. Asimismo, los cambios en los criterios diagnósticos tienen como objetivo principal permitir al clínico diagnosticar precozmente la EA. Por tanto, existe un gran interés en la detección de los primeros déficits cognitivos de la EA o DCL debido a EA,[4] es decir, antes que repercuta en las AVD del paciente y le convierta en dependiente de terceras personas.

3.1.3. Criterios para la investigación del DCL

En la última década se han publicado tres artículos que proponen los criterios diagnósticos en la investigación de la EA en los sujetos con DCL: los criterios del *International Working Group* (IWG)-1,[51,52] los IWG-2 [53] y los criterios NIA-AA. [4] En común, los tres grupos de criterios incluyen el uso de biomarcadores de patología

EA para aumentar el nivel de confianza respecto a que esos sujetos tengan como causa subyacente al DCL la EA. Sin embargo, difieren en la definición del DCL así como en la anormalidad del biomarcador (Tabla 1). [181,182] Para identificar la EA prodrómica en la práctica clínica un reciente estudio[182] propone el uso tanto de biomarcadores de βA como de daño neuronal siguiendo los criterios NIA-AA.[4] Además, para los ensayos clínicos ellos consideran seleccionar los sujetos del grupo con alta probabilidad de EA del NIA-AA[4] o del grupo EA prodrómico del IWG-2.[53] Estos nuevos criterios se fundamentan en la evidencia de que el proceso fisiopatológico de la EA suele comenzar años, e incluso décadas, antes de las primeras manifestaciones clínicas de la enfermedad. De esta forma existiría un retraso temporal entre la acumulación de placas βA, que parece constituir el primer signo anatomopatológico de la EA y el desarrollo de los primeros síntomas clínicos. [82,83,86-88] Así, entre las personas con DCL la prevalencia de depósitos βA positivo aumentaría con la edad de los 50 a los 90 años, así como ser portador del alelo ε4 del gen *APOE* y la presencia de deterioro cognitivo.[89]

Actualmente, la búsqueda de nuevas dianas terapéuticas se dirige a las fases preclínicas en la que el paciente es cognitivamente asintomático, pero algún biomarcador indica "un estado de riesgo para desarrollar la EA" así como a las fases prodrómicas de la EA, en la que se producen los primeros cambios clínicos, como pueden ser cambios leves en memoria u otras funciones cognitivas, en ausencia de repercusión en sus AVD. Por lo tanto, es necesario fomentar la investigación para detectar precozmente la EA y, cuando exista un tratamiento preventivo o curativo, su intervención precoz, con un seguimiento clínico exhaustivo y la mejora de la calidad de vida de las personas afectadas y de sus cuidadores[35].

Tabla 1. Clasificación según los criterios IWG-1, IWG-2 y del *National Institute on Aging–Alzheimer's Association* (NIA-AA) (adaptado de Visser et al., [181] y Vos et al., [182]).

DIAGNÓSTICO	**DEFINICIÓN**
IWG-I (2007)[52]	**Criterios de investigación para la EA prodrómica**
EA prodrómica	• Deterioro cognitivo en cualquier dominio cognitivo, • al menos un biomarcador de EA anormal. Estos pueden ser: -**Topográficos:** atrofia del lóbulo temporal medial en RMN o hipoperfusión en la corteza parieto-temporal en el PET-FDG. -**Patofisiológicos:** bajos niveles de amiloide –b1-42 en LCR, altos niveles de Tau en el LCR o aumento en PET de la captación de amiloide.
IWG-2 (2014)[53]	
EA no prodrómica	• Deterioro cognitivo en cualquier dominio, • βA1-42 en LCR y/o Tau normal o • PET de βA normal.
EA prodrómica	• Deterioro cognitivo gradual y progresivo[a] en memoria episódica de tipo hipocámpico[b] aislado o acompañado de otras alteraciones cognitivas/conductuales sugestivas de DCL o de un síndrome de demencia. Disminución de βA1-42 en LCR y/o aumento de Tau o • Aumento de la captación de βA en PET.
NIA-AA (2011)[4]	**Criterios clínicos y de investigación del DCL debido a EA (en relación al número de biomarcadores anormales)**
Grupo con baja probabilidad de EA	Deterioro cognitivo en cualquier dominio, marcadores de amiloide y daño neuronal normales.
Grupo con alta probabilidad de EA	Deterioro cognitivo en cualquier dominio, marcadores de amiloide y daño neuronal anormales.
Grupo conflictivo IAP (*isolated amyloid pathology group*)	Deterioro cognitivo en cualquier dominio, niveles βA anormales y marcadores de daño neuronal normales.
Grupo conflictivo SNAP (*suspected non-Alzheimer pathophysiology group*)	Deterioro cognitivo en cualquier dominio, niveles βA normales y marcadores de daño neuronal. anormales.
Grupo con probabilidad intermedia de EA	Deterioro cognitivo en cualquier dominio, un marcador testeado y anormal.
Grupo inconcluso/no informativo	Deterioro cognitivo en cualquier dominio, un marcador testeado y normal.

Marcador de amiloide= βA 1-42 en Líquido céfalo raquídeo (LCR); marcador de daño neuronal= valores de Tau en LCR/ valores de atrofia en Resonancia Magnética Nuclear (RMN) del lóbulo temporal medial/volumen de hipocampo/ Tomografía por Emisión Positrones Flúor-18 Flúordeoxiglucosa (PET-FDG), deterioro cognitivo definido como puntuación Z ≤1,5; [a] gradual y progresivo: empeoramiento de la memoria informado por el paciente o un informante (≥ 6 meses) [b] Síndrome amnésico de tipo hipocámpico: evidencia objetiva de deterioro significativo del rendimiento en una prueba de memoria episódica, a través de una prueba específica para la EA, como el recuerdo con claves con pruebas con control de los procesos de fijación.

3.2. La Enfermedad de Alzheimer (EA)

La EA es la causa más común de demencia,[21] representando el 50-60% de todos los casos de demencia en Europa**.** En España, se calcula que existen unos 400.000 afectados con la EA, muchos de ellos aún sin diagnosticar. La EA es más frecuente en mujeres y la edad constituye uno de los principales factores de riesgo. Se estima que en el año 2.050 uno de cada tres españoles tendrá más de 65 años. Por lo tanto, habrá aproximadamente un millón de pacientes con demencia, de los cuales entre el 60 y el 80% será de tipo Alzheimer.[145] Así pues, debido a que en la actualidad se está produciendo un envejecimiento de la población causado por el aumento de la esperanza de vida y la disminución de la mortalidad, la Organización Mundial de la Salud (OMS) ha avisado de las posibles consecuencias de este hecho y anima a los gobiernos a tomar medidas que reduzcan el impacto socio-sanitario de esta patología. Por este motivo, la OMS considera la demencia un objetivo prioritario para la salud pública global.[186]

Los síntomas de la EA como una entidad nosológica definida fueron descritos por Emil Kraepelin,[96] mientras que la neuropatolología característica, tal como la presencia de ovillos neurofibrilares intraneuronales y de placas seniles (placas neuríticas) en el espacio interneuronal, fue observada por primera vez por Alois Alzheimer en 1906.[1,2,18] La patología de la EA se caracteriza por la muerte neuronal progresiva, que comenzaría años o incluso décadas, antes de la aparición de los síntomas clínicos.

Los signos neuropatológicos de la EA son las placas extraneuronales consistentes en la deposición de la proteína βA y los ovillos neurofibrilares intraneuronales compuesta por la proteína tau (ptau) hiperfosforilada.[27]

La principal teoría subyacente a la fisiopatología de la EA es la hipótesis de la cascada amiloide, que postula que los cambios en la homeostasis de la βA conducen a la formación de placas de amiloide y ovillos de tau, lo que provoca la pérdida de células neuronales y sinapsis.[76] Finalmente, este proceso de neurodegeneración se traduce en disfunción cognitiva.

Respecto a los síntomas clínicos característicos de la EA [31,95] el deterioro cognitivo es lo más prominente.[65,188] El deterioro de la memoria episódica verbal, es el síntoma más importante de ambos síndromes,[59,73,120,121,142] la demencia tipo Alzheimer y la fase prodrómica denominada DCL,[4] que suele ser la manifestación clínica más temprana de la EA. El DCL es un síndrome caracterizado por el deterioro de uno o más dominios cognitivos, pero sin interferencia con las actividades de la vida diarias normales.[138,142] El diagnóstico temprano de la EA es importante, ya que el consenso actual entre los científicos es que la prevención debe iniciarse en una frase temprana en individuos con mayor riesgo. A medida que avanza la enfermedad, se producen alteraciones en otros dominios cognitivos tales como las funciones ejecutivas, el lenguaje, visuopercepción y praxis.[93] Los cambios conductuales y afectivos y las alteraciones en las AVD culminan en la demencia.

En las últimas décadas, los avances en la investigación sobre la EA, tanto a nivel clínico como biológico, ha requerido la revisión de los criterios diagnósticos del *National Institute of Neurological and Communicative Disorders and Stroke* (NINCDS) y el *Alzheimer's Disease and Related Disorders Association* (ADRDA)[113] y los del Manual de diagnóstico y estadístico DSM-IV[12] utilizados en los últimos 30 años para

la mayoría de los ensayos clínicos así como estudios de investigación. Así pues, con el objetivo de ayudar al clínico a realizar el diagnóstico precoz de la EA, en el año 2011 se publicaron en la revista *Alzheimer's & Dementia* los nuevos criterios diagnósticos NIA-AA para describir la EA y el DCL debido a EA. [4]

3.2.1. Criterios clínicos para la demencia

En la actualidad, en todos los entornos clínicos, los criterios básicos para el diagnóstico de la demencia [114] con independencia de la causa y del espectro de gravedad, que va desde la fase más incipiente hasta la más grave, siguen los nuevos criterios, abarcando los siguientes síntomas cognitivos o de conducta:

1) Interferencia con la capacidad de funcionar en el trabajo o en las costumbres y actividades sociales; 2) disminución de los niveles previos de funcionamiento y la realización de tareas; 3) no se explican por un delirio o trastorno psiquiátrico mayor; 4) el deterioro cognitivo se detecta y se diagnostica a través de la combinación de: la historia clínica del paciente y la información aportada por un acompañante así como de la evaluación cognitiva objetiva del estado mental y pruebas neuropsicológicas; 5) el deterioro cognitivo o conductual implica un mínimo de dos de los siguientes dominios: a) alteración de la capacidad para adquirir y recordar nueva información; b) deterioro del razonamiento y manejo de tareas complejas, juicio erróneo; c) deterioro de las capacidades visuoespaciales; d) alteraciones funcionales del lenguaje (hablado, leído, escrito) y e) cambios en la personalidad, la conducta o el comportamiento.

3.2.1.1. Criterios clínicos para la demencia tipo Alzheimer probable

Los principales criterios clínicos[114] que un paciente debe cumplir para ser diagnosticado de demencia tipo Alzheimer probable son los criterios para demencia descritos anteriormente y además, presentar las siguientes características: A) Inicio insidioso: los síntomas tienen un inicio gradual a lo largo de meses o años, no repentina en horas o días. B) Claros antecedentes de deterioro de la cognición en la información aportada por un informador fiable y C) los déficits cognitivos son evidentes en la historia y el examen en una de las siguientes presentaciones:

a) Presentación amnésica: Es el síndrome de presentación más común de la demencia tipo Alzheimer. Los déficits deben incluir el deterioro en el aprendizaje y en el recuerdo de la información recién aprendida. Debe ser también evidente la disfunción cognitiva en por lo menos otro dominio cognitivo, tal como se define en los criterios clínicos de la demencia.

b) Presentación no amnésica que a su vez puede ser: Afásica: Los déficits más importantes conciernen a la dificultad para encontrar las palabras, pero déficits en otros dominios cognitivos deben estar presentes. Visuoespacial: El más destacado es el déficit en la cognición espacial, incluidos los objetos, agnosia visual, alteración del reconocimiento de rostros, simultagnosia y alexia. Déficits en otros dominios cognitivos deben estar presentes. La disfunción ejecutiva: Los déficits más importantes conciernen a dificultades en el razonamiento, el juicio y la resolución de problemas. Déficits en otros dominios cognitivos deben estar presentes.

El diagnóstico de EA probable no debería ser aplicado cuando:

(a) Exista evidencia sustancial de enfermedad cerebrovascular definida por historia de un accidente vascular cerebral (AVC) relacionado temporalmente con la aparición o empeoramiento del deterioro cognitivo o la presencia de infartos múltiples o extensos o severa hiperintensidad en la sustancia blanca (b) características principales de la DCLewy además de la demencia en sí misma o (c) características prominentes de la DFT variante de conducta (vc) o (d) características prominentes de Demencia Semántica (DS), de Afasia Progresiva Primaria (APP) o variante agramática o (e) pruebas de una enfermedad neurológica, médica no neurológica asociada, comorbilidad o uso de medicación que podría tener un efecto sustancia sobre la cognición.

3.2.1.2. Criterios clínicos para la demencia tipo Alzheimer probable con aumento del nivel de certeza

La Demencia tipo Alzheimer probable con el declive documentado[114] comporta la evidencia de deterioro cognitivo progresivo evidenciado en posteriores evaluaciones sobre la base de datos de un informador y pruebas cognitivas. Las personas que cumplen los criterios clínicos básicos para la EA probable, con deterioro cognitivo documentado, aumenta la certeza de la enfermedad, pero no aumenta específicamente la certeza de que el proceso fisiopatológico sea debido a la EA.

3.2.1.3. Criterios clínicos de la EA probable en un portador de mutación genética

En las personas que cumplen los criterios clínicos básicos para la EA probable, la

evidencia de una mutación genética causante (*APP*, presenilina 1 -*PSEN1*- o presenilina 2 -*PSEN2*-), aumenta la certeza de que la condición es causada por la patología EA.[114] Ser portador del alelo ε4 del gen de la *APOE* no se considera suficientemente específico para ser considerado dentro de esta categoría.

3.2.1.4. Demencia tipo Alzheimer posible

El diagnóstico de la demencia tipo Alzheimer posible [114] debe hacerse en cualquiera de las circunstancias mencionadas en los párrafos siguientes:

1) Curso atípico: cumple los criterios clínicos básicos en términos de la naturaleza de los déficits cognitivos de la demencia tipo Alzheimer, pero o bien presenta un inicio repentino de deterioro cognitivo o muestra insuficientes detalles en la historia clínica, la documentación cognitiva objetivada o en el declinar progresivo.

2) Biomarcadores negativos: cumple criterios clínicos de demencia tipo EA, pero los biomarcadores (LCR, imagen estructural o funcional) no apoyan el diagnóstico.

3) Presentaciones etiológicas mixtas: etiológicamente, la presentación mixta reúne todos los criterios clínicos para la EA, pero hay evidencia de enfermedad cerebrovascular concomitante, (a) historia de un AVC relacionado temporalmente con la aparición o empeoramiento del deterioro cognitivo, la presencia de infartos múltiples o extensos o severa hiperintensidad de la sustancia blanca (b) características principales de la DCLewy, además de la demencia en sí misma o (c) características prominentes de

DFTvc o (d) características prominentes de DS, de APP o de APP variante logopénica; o (e) pruebas de una enfermedad neurológica, médica no neurológica asociada, comorbilidad o uso de medicación que podría tener un efecto sustancial sobre la cognición.

3.2.1.5. Demencia tipo Alzheimer probable con evidencia de proceso fisiopatológico de EA

La utilidad de los biomarcadores en la EA [114] ha sido ampliamente investigada, pudiéndose dividir en dos categorías:

1) Biomarcadores de depósitos de la proteína βA cerebral: a) Niveles bajos de βA-42 en LCR. b) una tomografía por emisión de positrones (PET) de amiloide positivo.

2) Biomarcadores de degeneración o lesión neuronal: a) Tau total y Tau Fosforilada (P-Tau) elevada en LCR. b) Disminución en PET de la captación de 18-fluorodesoxiglucosa (FDG), en la corteza témporo-parietal basal y lateral. c) Atrofia estructural desproporcionada en Resonancia Magnética (RM) Nuclear (RMN) en las regiones temporal medial, basal y lateral y la corteza parietal medial.

En las personas que cumplen criterios clínicos para la EA probable, la evidencia de biomarcadores incrementa la certeza de que la base fisiopatológica de la demencia clínica es debida a EA. Sin embargo, no podemos abogar por el uso de estos biomarcadores de forma rutinaria en el momento actual. Las limitaciones son:

1) Los criterios clínicos proporcionan muy buena precisión diagnóstica y de utilidad en la mayoría de los pacientes; 2) se requieren más investigaciones para asegurar que los criterios que incluyen los biomarcadores se han diseñado adecuadamente; 3) el acceso de los biomarcadores está limitado en mayor o menor grado a determinadas comunidades. Actualmente, el uso de los biomarcadores para obtener un diagnóstico fisiopatológico de certeza de la EA debe ser usado en tres circunstancias: estudios de investigación, ensayos clínicos y como herramienta clínica opcional cuando lo considere oportuno el profesional. Los resultados de las pruebas de los biomarcadores pueden clasificarse en tres categorías: claramente positivos, claramente negativos e indeterminados.

3.2.1.6. Demencia tipo Alzheimer posible con evidencia de proceso fisiopatológico de EA

Esta categoría se refiere a aquellas personas que cumplen criterios clínicos [114] para la demencia tipo no EA, pero que muestran evidencias de EA mediante biomarcadores o neuropatología. Se deberán incluir a aquellas personas que aun cumpliendo criterios clínicos de enfermedad por Cuerpos de Lewy o algún subtipo de Degeneración Lobar Frontotemporal (DLFT), muestran un biomarcador para la EA positivo o el estudio neuropatológico cumple los criterios de EA.

3.2.1.7. Demencia debida a EA fisiopatológicamente probada

El diagnóstico fisiopatológico probado de la EA [114] debería ser aplicado si el paciente cumple criterios clínicos y cognitivos de EA y el examen neuropatológico, usando los actuales criterios internacionales, demuestran las características patológicas de la EA.

3.3. Neuropsicología asociada a la demencia incipiente

El conocimiento de las características neuropsicológicas propias de la transición entre la normalidad propia del envejecimiento y los déficits de una enfermedad neurodegenerativa progresiva es crucial para poder detectar el desarrollo incipiente de un síndrome demencial. En el caso de la EA, en el *continuum* desde el funcionamiento cognitivo preservado hasta la demencia avanzada existirían estadios intermedios, cada uno de los cuales se asociaría a un nivel de funcionalidad. Así, a mayor intensidad del deterioro cognitivo se produciría una mayor interferencia con la funcionalidad del sujeto en sus AVD. [138] En España, las pruebas neuropsicológicas disponibles generalmente han sido adaptadas y validadas de las versiones originales reconocidas internacionalmente a nuestra población diana. Proyectos como el de *Estudios Normativos Multicéntricos Españoles* (NEURONORMA) han facilitado la validación de pruebas escritas originalmente en inglés para la población española.[130-132,134-136,151] Asimismo, algunas baterías neuropsicológicas han sido adaptadas y validadas para disponer de los valores normativos ajustados por edad y escolaridad.[9,107, 133,148] Todas estas pruebas fueron normalizadas mediante su administración a una muestra de individuos cognitivamente sanos representativa de la población española.

Una óptima clasificación de los sujetos con DCL, mediante baremos de nuestra población diana, contribuirá a detectar los perfiles cognitivos con un riesgo potencial de conversión a demencia. A nivel metodológico, para establecer los límites entre una función cognitiva preservada versus alterada es importante incluir grupos de pacientes con una condición patológica conocida (por ejemplo, pacientes con demencia leve) que permitirá el cálculo de los valores de sensibilidad (probabilidad de detectar un caso con

demencia) y especificidad (probabilidad de detectar un caso sin demencia) de la puntuación de un test. En España, los puntos de corte están disponibles para algunos test de cribaje (MMSE;[22] Test de los 7-Minutos[46]) así como para algunas pruebas de memoria (*Memory Impariment Screen;* [25] Test de Memoria -T@M-).[150] No obstante existen pocos estudios que evalúen el rendimiento cognitivo de las personas con DCL-a y DCL-na mediante baterías neuropsicológicas completas (p.ej., Batería Neuropsicológica de Fundació ACE –NBACE-) validadas en España y con baremos actualizados, proporcionando las puntuaciones estandarizadas, los percentiles y los puntos de corte (por rangos de edad y escolaridad) de cada una de las pruebas neuropsicológicas comprendidas, para facilitar la detección de demencia en población española.[8,9]

3.3.1. Neuropsicología de la EA prodrómica

Puesto que el DCL-a puede constituir la fase prodrómica de la EA, es crucial disponer de herramientas sensibles capaces de discriminar entre las personas que desarrollarán demencia de las que se mantendrán estables en el tiempo o normalizarán sus capacidades cognitivas. [109,129]

La mayoría de los pacientes con EA prodrómica muestran una alteración de la memoria episódica producida por una disfunción de la capacidad de almacenamiento de nueva información[47] caracterizada por una alteración de la memoria a largo plazo sin beneficio de ayudas externas, tales como tareas de memoria de reconocimiento o de facilitación con claves semánticas.

Aunque en la EA el deterioro de la memoria episódica es el síntoma más prominente en su fase prodrómica,[51,59,73,120,121,142] una de las limitaciones más frecuentes de los estudios sobre DCL es que la prueba de memoria carece de una tarea de memoria de reconocimiento.[28,70] Es importante determinar el patrón de alteración de la memoria, distinguiendo entre: 1) la alteración de la evocación libre (con preservación del rendimiento en la tarea de reconocimiento), normalmente asociada a una disfunción del circuito fronto-subcortical prefrontal dorsolateral[45] o 2) la alteración del almacenamiento de la información (y con alteración del rendimiento en la tarea de reconocimiento), asociada a una disfunción del lóbulo temporal medial (córtex entorhinal/perirrinal e hipocampo).[190]

Un peor rendimiento en el reconocimiento verbal se ha encontrado relacionado con una mayor atrofia del córtex entorhinal, demostrando ser sensible a la EA.[37,48,62,190] No obstante, en las fases iniciales los sujetos con un buen rendimiento en pruebas sensibles a las funciones ejecutivas aún podrían mostrar una relativa preservación en las pruebas de memoria de reconocimiento.[69] Así, en las fases prodrómicas de la EA, se esperará que los sujetos con preservación de las funciones ejecutivas estén compensando la alteración incipiente de la retención verbal, pero cuando el rendimiento en memoria de reconocimiento esté alterada, estará indicando que los pacientes se encuentran en una fase más avanzada de la enfermedad. Además, un estudio reciente[7] que comparó los datos basales neuropsicológicos y de perfusión cerebral (SPECT) entre los sujetos con DCL-a que convirtieron a EA y los que se mantuvieron estables a lo largo de dos años, mostró que los sujetos que desarrollaron demencia tipo Alzheimer obtuvieron un peor rendimiento en la prueba de memoria de reconocimiento verbal, mientras que no se encontraron diferencias estadísticamente significativas en el SPECT. En el seguimiento

a los cuatro años, aquellos que desarrollaron EA rindieron peor en las pruebas de memoria visual a largo plazo y fluencia verbal semántica. Asimismo, aunque la presencia de hipoperfusión cerebral en el giro postcentral y región basal cerebral, fueron indicadores de riesgo, la velocidad del declive desde el DCL a la EA se relacionó únicamente con el grado de deterioro en memoria. [6]

La progresión hacia la EA también puede ser precedida por diferentes procesos cognitivos disfuncionales y no únicamente por la alteración de la memoria. De hecho, raramente se encuentran sujetos con DCL-a-ud[142] tras la administración de una batería neuropsicológica exhaustiva, ya que normalmente muestran otras alteraciones cognitivas, tales como atencionales, ejecutivas y/o visuoperceptivas complejas, [5,7,56,127,175] incluso varios años antes del inicio de la demencia.[61]

Estudios recientes han demostrado que algunas pruebas cognitivas sensibles a la memoria episódica y las funciones ejecutivas (p.ej.: *Trail Making Test*) son capaces de predecir la conversión de DCL a demencia tipo Alzheimer con un nivel de precisión similar al que proporcionan métodos mucho más complejos y costosos, tales como la cuantificación de regiones cerebrales a partir de imágenes de RM (p.ej.: córtex entorhinal, hipocampo), pruebas de neuroimagen funcional (p.ej.: hipometabolismo cerebral mediante PET-FDG[38] ó los niveles de LCR (p.ej.: tau fosforilada, amiloide-β-42).[49,50,60,128] De hecho, se ha encontrado que al añadir las pruebas complementarias incrementa poco el valor predictivo que ya tenían las pruebas neuropsicológicas,[49,50,60] lo cual podría ser debido a la alta correlación existente entre las diferentes técnicas.

Tal y como sugirieron Weiner et al.[184,185] en su revisión, aunque los mejores predictores de conversión a demencia son el resultado de la combinación de diferentes técnicas (tales como datos de LCR, RM, PET-FDG, *APOE*-ε4 y los test cognitivos), los valores predictores de conversión a demencia no son óptimos. Por tanto, para conseguir una precisa discriminación entre los sujetos DCL conversores a demencia versus no conversores se requiere una mejora en la precisión de la clasificación diagnóstica del DCL. Así, el riesgo de conversión a demencia del DCL depende en gran parte de la definición aplicada de DCL [102,103,138] y de sus criterios neuropsicológicos específicos. [26] Una integración de los perfiles neuropsicológicos, genéticos, clínicos y los biomarcadores podría mejorar notablemente la capacidad predictiva.

3.4. Factores genéticos asociados a la demencia incipiente desde el DCL

Desde el punto de vista genético, se ha estimado que la EA de comienzo tardío (LOAD de *late-onset AD*) tiene una heredabilidad entre el 60 y el 80% incluso para los casos no familiares[189] y un patrón de herencia poligénico. Por su parte, la EA de comienzo precoz (con inicio en sujetos menores de 65 años, EOAD de *early-onset AD*) tiene una heredabilidad calculada de casi el 100% [l.C. 92%-100%]. Su modo de herencia es, predominantemente, autosómico recesivo.[189]

Desde el punto de vista molecular, la identificación de la variación genómica asociada a la EA es un gran reto tecnológico. Empleando técnicas clásicas de secuenciación exónica en tres genes detectados mediante estudios de ligamiento o bien deducidos como candidatos (*APP, PSEN1 y PSEN2*), se ha resuelto la mitad de los casos de transmisión autosómica dominante de EA. No obstante, estas mutaciones sólo explican entre un 2 y un 10% de los casos de EOAD y prácticamente ningún caso de LOAD.

Otro gran reto técnico es el aislamiento de los genes involucrados en los casos de EA de comienzo tardío (LOAD) y su aplicación para la identificación de sujetos afectados ligeramente (DCL), que aún no convirtieron a demencia, pero que convertirán con alta probabilidad (en ellos se podría realizar prevención secundaria). El problema de LOAD es que casi todos estos genes y exposiciones asociados a EA son desconocidos. No obstante, el desarrollo imparable de las tecnologías genómicas van aportado los primeros loci vinculados a LOAD. En concreto en la era pre-*Genome-wide association study* (GWAS) se identificó *APOE*,[41] que se conoce desde hace más de 15 años pero

ningún otro locus había sido aceptado universalmente por la comunidad científica hasta el año 2009.

Los rastreos completos del genoma tipo GWAS, utilizando micromatrices de polimorfismos de un solo nucleótido (SNPs) que cubren todo el genoma, las estrategias de meta-análisis e imputación del genoma completo y, más recientemente, el análisis de exomas, están catapultando rápidamente la identificación de las bases genéticas de la EA. Gracias al esfuerzo cooperativo de diversos consorcios científicos internacionales como el *Alzheimer's Disease Genetics Consortium* (ADGC), el *European Alzheimer's Disease Initiative* (EADI), el *Genetic and Environmental Risk in Alzheimer's Disease* (GERARD), el *Cohorts for Heart and Aging in Genomic Epidemiology* (CHARGE) o el *International Genomics Alzheimer's Project* (IGAP), hoy conocemos veintitrés regiones genómicas "incontrovertiblemente" asociadas a EA.[100] Estos loci se consideran incontrovertibles porque se asociaron a LOAD con un valor de significación estadística (p) inferior a 5x10E-8 e integraron la información, al menos, de cuatro réplicas de estudios de casos y controles en poblaciones independientes. La estrategia de meta-análisis de los resultados de los GWAS (meta-GWAS) ha supuesto un salto cualitativo muy importante en cuanto a la fiabilidad de las señales seleccionadas.

Por su parte, la secuenciación completa de la secuencia codificante de ADN germinal (exones) de series de casos y controles de diversas poblaciones y su posterior meta-análisis, ha permitido la identificación de una variante codificante rara en el gen *TREM2, R47H,* asociada a LOAD con una penetrancia equivalente al alelo *APOE-*

ε4.[74,90] Se efectuó la validación y meta-GWAS que localizó los loci *BIN1* y *EXOC3L2* y,[172] se verificaron cuatro más, *PICALM*, Clusterin/*APOJ*, *MS4A* y *CR1*, en un GWAS independiente.[13] Del mismo modo, se han realizado numerosas replicaciones en la población española. Más recientemente, se ha identificado una nueva señal GWAS significativa para LOAD en colaboración con el consorcio CHARGE. El nuevo locus, *ATP5H/ KCTD2*, ha emergido realizando un nuevo meta-GWAS con más de 24.000 individuos y seis réplicas independientes ($p= 3.8 \times 10^{-9}$).[23]

Es conveniente finalizar el estado del arte sobre la genética de EA mencionando que el esfuerzo unificado de los grupos europeos y norte-americanos más importantes (el consorcio IGAP) ha permitido configurar un nuevo gran meta-GWAS con una muestra de 74.046 sujetos y que ha doblado el número de loci vinculados a EA. [100] La identificación de estos nuevos genes y la información concreta de los SNPs asociados, se ha publicado muy recientemente, de forma que además de los loci conocidos del GWAS, tales como *CD2AP*, *EPHA1*, *ABCA7*, *CD33*, se han identificado 11 nuevos loci, *HLA-DRB5-HLA-DRB1, PTK2B, SORL1, SLC24A4-RIN3, INPP5D, MEF2C, NME8, ZCWPW1, CELF1, FERMT2 y CASS4*.[100]

Así pues, contamos con un total de 23 loci incontrovertiblemente vinculados a EA. Desafortunadamente, el hecho de localizar estos genes no supone ninguna variación en el manejo clínico de los pacientes EA. Es decir, esta información debe ser procesada adecuadamente para que impacte en el diagnóstico, tratamiento y pronóstico de los sujetos con EA en el futuro. Más importante, esta información puede ayudar a

transformar el manejo de sujetos con DCL en riesgo de desarrollar demencia (estrategias de prevención secundaria mencionadas con anterioridad). La población de riesgo, diana del presente estudio, es la de los sujetos con DCL.

No obstante, las clasificaciones existentes de DCL no han tenido en cuenta el perfil genético global de los individuos, ni han integrado los nuevos conocimientos obtenidos de la investigación de los estudios genéticos en los pacientes con EA. Existen, por tanto, pocos estudios al respecto, su tamaño muestral es relativamente escaso y, desde luego, ninguno de ellos integra los 23 loci.

El estudio Rotterdam demostró que los loci disponibles hasta 2011 eran insuficientes para predecir el riesgo de conversión a demencia.[179] Hallazgos preliminares del grupo español liderado por el Dr. Onofre Combarros apuntan, sin embargo, a que la combinación de alelos de riesgo podría tener valor en la variable conversión a demencia.[159] En cambio, un estudio de rastreo completo del genoma (GWAS) de pacientes DCL determinó que los genes de susceptibilidad conocidos no predecían la conversión a EA. [80]

Vistos estos hallazgos controvertidos, en la presente tesis se plantea una estrategia de meta-análisis, añadiendo los nuevos loci no incluidos en estudios anteriores, para tratar de profundizar en la utilidad clínica de estos marcadores genéticos. Para conocer el impacto de los nuevos genes en el diagnóstico de EA, se debe analizar su papel individual, locus por locus y colectivamente (combinaciones multilocus o multigénicas) en el riesgo de conversión a EA de sujetos con DCL. La identificación de los loci más

importantes de conversión a EA es útil: i) Es una estrategia independiente de validación de los hallazgos obtenidos en los estudios de caso/control; ii) Aporta nuevos datos sobre los patrones de conversión a EA y otras demencias en pacientes con DCL y su variabilidad fenotípica; iii) Permitirá priorizar los loci más interesantes para realizar posteriormente desarrollos de utilidad clínica o seleccionar aquellas funciones más prometedoras con objeto de aislar la variante causal e identificar el mecanismo molecular subyacente que permita diseñar abordajes terapéuticos más racionales. Ello permitirá la implantación de una actuación médica más racional y adecuada a cada caso en el futuro.

3.5. Correlación fenotipo-genotipo

En las próximas décadas se esperan avances tanto en neuropsicología y neurociencia como en genética y neuroimagen. La finalización del Proyecto Genoma Humano, ha impulsado el desarrollo de sistemas computacionales de alto rendimiento para acelerar el avance en el conocimiento de los fundamentos moleculares de las enfermedades humanas y, consecuentemente, conocer la base molecular de estrategias más eficaces para prevenir, diagnosticar precozmente y tratar las enfermedades. Conocer la secuencia completa del genoma humano puede tener mucha relevancia en cuanto a los estudios de biomedicina y genética clínica. Sin embargo, descubrir toda la secuencia génica de un organismo, es decir, su genotipo y que podemos definir como el conjunto de genes que presenta un individuo no nos permite inferir por completo el fenotipo asociado, es decir, el conjunto de caracteres morfológicos, funcionales, bioquímicos, conductuales, etc., que presenta un ser vivo.

Aunque los fenotipos alterados (p.ej.: alteración de la memoria episódica) representan algunas de las manifestaciones más fiables de algunos factores genéticos, la investigación sistemática de relaciones entre los genotipos portados y sus expresiones fenotípicas están todavía en sus inicios. De hecho, en los últimos años se han propuesto las pruebas neuropsicológicas, sobre todo de memoria, como endofenotipos complejos de la EA.[105] Los endofenotipos son características cuantitativas que están relacionadas con una patología de base. El estudio de endofenotipos de la EA, simples y económicos, pueden permitir refinar e identificar los individuos con EA incipiente o con riesgo de desarrollarla en un futuro próximo. Esto permitiría optimizar recursos sanitarios y reducir el coste económico (mejora del coste-eficacia).

Determinar los genes que afectan a la expresión de endofenotipos importantes para la patología en estudio puede ayudar a diseccionar la base molecular de patologías complejas como la EA.[44] Asimismo, el desarrollo de bases de datos fenotípicas está muy por detrás del rápido avance de las bases de datos genómicas. Estudios recientes se han centrado en el estudio de la relación fenotipo-genotipo, mediante aproximaciones computacionales de alto rendimiento[104] y mediante las ontologías cognitivas o estructuras jerárquicas de clasificaciones por entidades, las cuales son necesarias para maximizar el potencial de realización de tales avances.[19]

Hasta ahora, no obstante, las clasificaciones de DCL descritas en la literatura, no han tenido en cuenta el perfil genético de los individuos, no han integrado los nuevos conocimientos obtenidos de la investigación de los biomarcadores en los pacientes ni se han utilizado las técnicas más modernas de análisis bioinformático para reclasificar fenotípicamente a los pacientes y crear nuevos paradigmas para la identificación de sujetos con DCL en riesgo de conversión a demencia.

La presente tesis aporta a los estudios anteriores un análisis computacional multivariado de predicción de conversión del DCL a EA (u otras demencias), a través de técnicas estadísticas, mediante la combinación de diferentes variables (neuropsicológicas, biomarcadores en neuroimagen y genéticas) con el objetivo de conseguir diagnosticar la EA, u otras demencias, en sus estadios prodrómicos/predemencia, es decir, antes de lo que los criterios actuales permiten.

4. HIPÓTESIS DE TRABAJO Y OBJETIVOS

El reto diagnóstico actual en las unidades de memoria consiste en tratar de identificar, entre los sujetos con DCL, a aquellos que rápidamente evolucionarán a EA. Esto es necesario para lograr diferenciar los individuos que tienen una EA prodrómica de los que sufren otros procesos (patológicos o incluso vinculados al envejecimiento natural), habitualmente no degenerativos y con un pronóstico y manejo clínico diferente. Identificar a los sujetos DCL que convertirán a EA permite ofrecer a estos sujetos, que ya padecen la enfermedad en estadio prodrómico, su incorporación a programas de terapias experimentales y el establecimiento de un programa de actuaciones en su entorno social, económico y sanitario que permita, tanto al paciente como a su familia, afrontar el proceso patológico de la mejor manera posible.

Ahora mismo toda nuestra potencia predictiva se basa en los resultados de algunas baterías neuropsicológicas, el empleo de biomarcadores en líquido cefalorraquídeo (niveles de Tau/p-Tau y amiloide-β42) y la identificación de patrones neuro-radiológicos empleando técnicas alta resolución como el PET-FDG o la RMN-DTI. A pesar de estos avances, las técnicas que usamos son de aplicación muy tardía. Además, con todo ello, no hemos logrado confeccionar un algoritmo que integre todas estas técnicas junto con los factores de riesgo genético conocidos. No se conoce adecuadamente la capacidad predictiva de demencia de los nuevos factores de riesgo genéticos identificados en la EA, no obstante, posee una alta heredabilidad que supera el 70%. Su relación con el resto de rasgos clínicos que definen la enfermedad, o con los resultados de las pruebas neuropsicológicas o de la neurorradiología de los pacientes con DCL se conoce menos aún. A largo de este proyecto daremos respuesta a varias de estas incógnitas. Planteamos la valoración de un modelo genético de conversión.

La hipótesis de la presente tesis consiste pues en que la construcción de un predictor genético nuevo, duplicando el número de genes relevantes de EA, mejorará las capacidades de predicción de conversión a demencia en sujetos con DCL sugeridas en estudios anteriores.

-Objetivos:

1. Determinar el riesgo de conversión a demencia, y detección de los principales factores de riesgo implicados, en una amplia muestra de pacientes con DCL.

2. Evaluar el efecto de una amplia gama de marcadores genéticos (tipo SNP derivados de GWAS) en sujetos diagnosticados de DCL, sobre la conversión a EA.

3. Identificar modelos predictivos univariantes y multivariantes de conversión a EA empleando técnicas estadísticas y estudios de supervivencia.

4. Replicar los SNPs significativos en series adicionales de DCL (en Alemania y Ámsterdam).

5. Identificar los elementos redundantes entre los marcadores genotipados a fin de priorizar los grupos de marcadores más poderosos desde un punto de vista predictivo.

6. Realizar un meta-análisis de los resultados más relevantes originales en estas series.

7. Analizar la asociación entre los patrones neuropsicológicos y los marcadores genéticos en una amplia muestra de pacientes con DCL clasificados en cuatro subtipos (amnésico/no amnésico x probable/posible).

5. MÉTODO

Esta tesis doctoral consta de cuatro estudios, tres de los cuales ya están publicados [57,58,99] y el cuarto está en revisión.

Sujetos

Estudio retrospectivo: Los datos fueron analizados a partir de una muestra de 550 sujetos diagnosticados de DCL en la Unidad de Diagnóstico de Fundació ACE, entre enero de 2006 y noviembre de 2011. Los criterios de inclusión fueron: una puntuación de 0,5 en la *Clinical Dementia Rating Scale* (CDR), [119] edad superior a 60 años, con escolaridad mínima equivalente a analfabetismo funcional, sin déficits auditivos ni visuales severos, incluyendo glaucoma y cataratas, con ADN disponible y genotipados de *APOE*. Sus historias clínicas fueron revisadas para clasificarles de acuerdo a los criterios de Petersen et al. [138,142] (amnésico y no amnésico; único y múltiple dominio) y teniendo en cuenta la clasificación de López et al. [102] (probable y posible). Así, los sujetos con DCL se dividieron en cuatro subtipos: DCL amnésico probable (DCL-a-Pr) (n= 115), no-amnésico probable (DCL-na-Pr) (n= 37), DCL amnésico posible (DCL-a-Ps) (n= 234) y no-amnésico posible (DCL-na-Ps) (n= 164) con afectación en un único o múltiples dominios. El DCL posible fue asignado sólo cuando se atribuyó a una etiología cerebrovascular o psiquiátrica (ansiedad o depresión).

Estudios prospectivos:

1. **Estudio con biomarcadores de neuroimagen:** Se analizó una muestra de sujetos con DCL-a-Pr a nivel de almacenamiento (n=20) y 39 controles sanos (CS), con

edad superior a 64 años, pertenecientes al estudio de Fundació ACE (ACE), reclutados entre julio de 2010 y enero de 2013. Los pacientes con DCL-a-Pr a nivel de almacenamiento cumplían los mismos criterios de inclusión que el estudio retrospectivo[102,103,138,142] y con un rendimiento alterado para su edad y nivel de escolaridad en el recuerdo diferido y reconocimiento verbal, de la Lista de Palabras de la prueba de Aprendizaje de la Escala de Memoria de la Wechsler-Tercera edición (WMS-III) (sin utilizar la lista de interferencia) [8,9] y el *Free and Cued Selective Reminding Test* (FCSRT). [30,132]

Los sujetos CS cumplían los siguientes criterios de inclusión: una historia clínica normal para su edad, sin signos ni síntomas neurológicos, no informaron problemas de memoria ni en otras funciones cognitivas, puntuación mayor de 25 en el MMSE,[22,64] rendimiento dentro de la normalidad para su edad y nivel de escolaridad en las pruebas de memoria episódica verbal, [8,9,30,132] una puntuación de 0 en la CDR;[119] y ninguno tuvo antecedentes familiares de demencia.

Todos con biomarcadores de neuroimagen disponibles, una RM estructural, un PET-FDG y un PET con el Compuesto B de Pittsburgh (N-Methyl-[11C]2-(4'-methylaminophenyl) 6 hydroxy- benzotriazol (PET-PIB). Además, se analizó la muestra de sujetos con DCL-a-Pr a nivel de almacenamiento (n=133) y 42 sujetos CS del estudio parental AB255. Estos pacientes que cumplieron los mismos criterios de inclusión que en el estudio ACE, fueron reclutados y evaluados en 19 centros clínicos y de investigación de la memoria en España, Italia, Suecia y Francia y fueron liderados por Araclon Biotech S.L. Todos con RM estructural y con PET-FDG disponibles.

La construcción de las puntuaciones de cinco dominios cognitivos en *Composites* Cognitivos así como las medidas del volumen de hipocampo ajustado (VHa) de la RM estructural, y las medidas del ratio del valor de captación estandarizado (SUVR) del PET-FDG, se replicaron en la base de datos parental AB255.

2. Correlación fenotipo-genotipo y asociación endofenotipo–genotipo: Se analizó una muestra ampliada hasta 1.250 sujetos que acudieron a la unidad de Diagnóstico de Fundació ACE, entre enero de 2006 y julio de 2013 y que fueron diagnosticados de DCL. [102,103,138,142] Estos pacientes cumplían los mismos criterios de inclusión que en el estudio retrospectivo. Todos se dividieron en los cuatro subtipos, tal y como se procedió en el estudio retrospectivo: DCL-a-Pr (n= 262), DCL-na-Pr (n= 76), DCL-a-Ps (n= 549) y DCL-na-Ps (n= 358) con afectación en un único o múltiples dominios. El DCL posible fue asignado sólo cuando se atribuyó a una etiología cerebrovascular o psiquiátrica (ansiedad o depresión).

Criterios diagnósticos: Todos los sujetos fueron reclutados y evaluados en Fundació ACE y todos los diagnósticos consensuados entre los neurólogos, neuropsicólogos y trabajadores sociales. Todos tuvieron al menos un seguimiento (rango: 6-68 meses y 12 a 36 meses) para el estudio retrospectivo y prospectivo respectivamente, y los diagnósticos de seguimiento se realizaron con pleno conocimiento de la clasificación anterior, así como de los antecedentes neurológicos y psiquiátricos. Se consideró que todos los pacientes tenían un deterioro cognitivo no suficiente como para justificar el diagnóstico de demencia. Se permitieron deficiencias menores en actividades complejas

de la vida diaria, pero no hubo informes de disminución de las capacidades intelectuales generales y todos los pacientes fueron autónomos en el momento de la inclusión.

Patrón de deterioro del dominio (PDD): criterios de DCL amnésico y no amnésico, único y múltiple dominio: El DCL se subclasificó como DCL-a-ud, DCL-a-md, DCL-na-ud y DCL-na-md cumpliendo los criterios de Petersen et al., [138,142] incluidas las quejas subjetivas de memoria, la cognición global preservada, rendimiento conservado en las actividades de la vida diaria, ausencia de demencia y un deterioro apreciable en función de la memoria, con o sin déficit en otros dominios cognitivos (DCL-a-ud o DCL-a-md).

Patrón de deterioro de la memoria (PDM): criterios del DCL amnésico de almacenamiento y recuperación: Los sujetos con DCL-a (DCL-a-ud y DCL-a-md) con una alteración de la memoria verbal a largo plazo y sin beneficio de las pruebas de reconocimiento fueron clasificados dentro del patrón de alteración de la memoria de “almacenamiento". Por el contrario, si obtuvieron beneficio del reconocimiento, fueron clasificados como afectos de un déficit de "recuperación".

Criterios del DCL Probable y Posible: Similar a la clasificación de López et al.,[102] pero extendido a los grupos DCL no amnésico (DCL-na-ud y DCL-na-md), todos los sujetos con DCL fueron reclasificados como DCL posible en presencia de comorbilidades que podrían explicar o contribuir a los déficits cognitivos, y se clasificaron como DCL probables cuando no había ninguna. Por lo tanto, los sujetos fueron clasificados como DCL-a-Ps cuando existía comorbilidad psiquiátrica, neurológica (es decir, enfermedad cerebrovascular, historia de traumatismo craneal,

encefalopatía, enfermedades infecciosas o discapacidades del desarrollo), enfermedades sistémicas que pudieran causar déficits cognitivos, o bien cuando no se disponía de suficiente información. En el presente estudio, para los grupos DCL-a-Ps y DCL-na-Ps, sólo se incluyeron los sujetos con enfermedad cerebrovascular y trastornos psicoafectivos (ansiedad o depresión). Los pacientes con antecedentes de otra enfermedad psiquiátrica (por ejemplo, trastorno bipolar) no fueron considerados en el análisis. Por el contrario, los sujetos fueron clasificados como DCL-a-Pr o DCL-na-Pr si no existían enfermedades neurológicas, psiquiátricas o sistémicas que pudieran explicar los déficits cognitivos objetivados.

Criterios de los DCL conversores y no conversores: Los sujetos que convirtieron a demencia a lo largo del estudio, como son EA;[113,114] DM (EA con enfermedad cerebrovascular asociada), DFT;[124,152,153] DV o DCLewy;[112,160] fueron clasificados como DCL convertidores. Por el contrario, aquellos sujetos que permanecieron estables durante el seguimiento fueron clasificadas como estables o DCL no convertidores. Por último, los pacientes que normalizaron su rendimiento cognitivo, aunque tuviesen quejas subjetivas de memoria (por ejemplo, 10 sujetos en el estudio retrospectivo), fueron excluidos del análisis.

Exploración clínica: Todos los participantes recibieron exámenes neuroconductuales estandarizados, incluyendo un examen neurológico, una evaluación neuropsicológica exhaustiva y una visita de trabajo social. La información sobre los factores de riesgo vascular (incluyendo la hipertensión, la hipercolesterolemia, la diabetes mellitus, los antecedentes de accidente cerebrovascular, la enfermedad cardíaca, y la historia familiar de demencia) fue proporcionada por los pacientes o sus cuidadores. Las imágenes por

RM, o más generalmente tomografía computarizada (TC), estaban disponibles para su revisión. Fueron requeridas varias manifestaciones de la enfermedad vascular significativa (por ejemplo, hipertensión, angina de pecho) y los hallazgos en la RM (o TC), asociados a la enfermedad cerebrovascular para una clasificación de DCL posible de etiología cerebrovascular.

Exploración Neuropsicológica: La batería neuropsicológica de Fundació ACE (NBACE),[8,9] se administró a todos los pacientes con DCL en Fundació ACE. La batería NBACE [8,9] incluye pruebas sensibles a la velocidad de procesamiento de la información, orientación, atención, capacidad de aprendizaje y memoria a largo plazo verbales, lenguaje, gnosias, praxias, funciones visuoespaciales y ejecutivas, incluyendo las siguientes pruebas: Orientación Temporal, Espacial y Personal; los subtest Dígitos Directos e Inversos, Cubos (abreviado de forma que de los ítems 6 al 9 se puntuó sólo la precisión (1 punto) sin una bonificación por tiempo) y Semejanzas (abreviadas a los primeros 10 ítems) de la Escala de Inteligencia de la Wechsler para Adultos-Tercera Edición (WAIS-III); la Lista de Palabras de la prueba de Aprendizaje de la WMS-III (sin utilizar la lista de interferencia); Repetición (2 palabras y 2 frases); Comprensión verbal (ejecución de 2 órdenes simples, 2 semi-complejas y 2 complejas, extraídas de la Alzheimer's Disease Assessment Scale-Cognitive (ADAS-Cog) y la batería de pruebas del Test Barcelona); La prueba del Test de Denominación de Boston, versión abreviada de 15 ítems; el test de Poppelreuter; los Relojes de Luria; el Test de los 15-Objetos; el subtest de Inhibición de respuestas automáticas del *Síndrome Kurtz test* (SKT); Fluidez verbal fonética (palabras que empiecen por 'P' durante un minuto); Fluidez verbal semántica ("animales" durante un minuto), y la versión española del Test del Reloj.

Exploración neuropsicológica en el estudio con biomarcadores de neuroimagen: Todos los sujetos del estudio ACE y AB255 fueron sometidos a una evaluación neuropsicológica incluyendo cinco dominios cognitivos: (1) Aprendizaje: Test de aprendizaje auditivo verbal de Rey (TAVR) [157] y ADAS-Cog [161] (únicamente el recuerdo inmediato en memoria); (2) Recuerdo Diferido: verbal en el TAVR [157] y ADAS-Cog [161] y no verbal (Figura compleja de Rey-Osterrieth (FCRO))[156]; (3) Velocidad de Procesamiento, Atención y Funciones Ejecutivas: Test Símbolo Dígito y span de Dígitos directos e inversos, [183] Test del Trazo (Trail Making Test (TMT)) (parte B-parte A) [155]; Fluidez verbal semántica ("animales" durante un minuto), [72] y Fluidez Verbal Fonética (palabras que empiecen por "P" durante un minuto);[15] Lenguaje: Test de Denominación de Boston [92] y ejecución de órdenes del ADAS-Cog;[161] y (5) Praxias: copia de la FCRO [156] y Cubos de la WAIS-III. [183]

Construcción de los *Composites* Cognitivos: Para la construcción de los cinco *Composites* Cognitivos se realizó un Análisis de Componentes Principales (ACP). Por lo tanto, se llevaron a cabo cinco ACP por separado, uno por cada uno de los siguientes dominios cognitivos: 1) Aprendizaje y 2) Recuerdo Diferido en Memoria, 3) Funciones Ejecutivas, 4) Lenguaje y 5) Praxias. La estabilidad del ACP se evaluó mediante la prueba Hotelling's T2.

Adquisición y análisis de la Neuroimagen: Todos los sujetos del estudio ACE fueron sometidos a una RM estructural, un PET-FDG y un PET-PIB dentro de los 30 días siguientes a la valoración neurológica y neuropsicológica. Todos los sujetos del estudio AB255 fueron sometidos a una RM estructural y a un PET-FDG. Los datos de imagen se analizaron utilizando el servidor de Fundació ACE para el Análisis de Neuroimagen.

Genotipado de *APOE* en el estudio retrospectivo: El alelo *APOE*-ε4 fue identificado con kits comerciales para *APOE* rs429358 (SNP112) y rs7412 (SNP158) de Roche Diagnostics (Alemania). Los alelos *APOE* fueron amplificados utilizando LightCycler Kit de Detección de Mutaciones *APOE* (Roche Diagnostics, Alemania) y se detectaron utilizando la tecnología de PCR *(polymerase chain reaction)* en tiempo real (480 LightcyclerR Sistema, Roche Diagnostics, Alemania) siguiendo las instrucciones del fabricante. Para comprobar la calidad de los resultados, los diferentes heterocigotos compuestos para *APOE*, SNPs fueron verificados en un laboratorio de investigación independiente. Sólo una muestra de sangre no se pudo genotipar en el estudio retrospectivo debido a que accidentalmente resultó dañada.

Genotipado en los estudios prospectivos:

Extracción y crio-preservación de ADN e integración de datos clínicos y genéticos: Los datos clínicos adscritos de cada muestra fueron procesados y organizados en bases de datos relacionales para su utilización tras la integración de las variaciones genéticas de cada sujeto. Las muestras sanguíneas obtenidas en Fundació ACE, se procesaron rutinariamente en el laboratorio de genética molecular. La extracción de ADN se realizó mediante procedimientos habituales (Sistema Maxwell 16, Promega). La genotipación se llevó a cabo mediante el sistema Sequenom. Usando este método, se analizaron diversos marcadores genéticos de interés en la EA. En concreto 40 SNPs derivados de GWAS, o meta-GWAS [13,100,172] se genotiparon en 3.326 sujetos con DCL pertenecientes a cuatro series independientes, la serie alemana: *The German study*

on Aging, Cognition, and Dementia in primary care patients (AgeCoDe, n=853) y *The German Dementia Competence Network* (DCN; n=812), la Fundació ACE en Barcelona, España (ACE, n=1245) y la *Amsterdam Dementia Cohort* (ADC, n=306). El proceso se realizó en el Departamento de Genómica en el Centro *Life & Brain*. Instituto de Genética Humana de la Universidad de Bonn (Alemania).

Análisis estadístico

Estudio retrospectivo: El análisis estadístico se llevó a cabo mediante el software SPSS para Windows (v18.0, SPSS Inc, Chicago, IL). Para comparar los datos demográficos, clínicos y genéticos entre los cuatro grupos, se realizó un Análisis de la Varianza (ANOVA) o Chi cuadrado para las variables cuantitativas y cualitativas, respectivamente. Para comparar las puntuaciones basales en las pruebas neuropsicológicas NBACE [8,9] entre los grupos DCL, se ejecutó un Análisis de la Covarianza (ANCOVA), ajustando las variables por género, edad y escolaridad. Cuando una variable resultó estadísticamente significativa, se realizaron comparaciones múltiples post-hoc de Bonferroni.

Para analizar los predictores de conversión a demencia, se llevaron a cabo estudios longitudinales utilizando los modelos de riesgos proporcionales de Cox, ajustados por género, edad y escolaridad, introduciendo las diferentes variables de acuerdo a los diferentes modelos considerados: variables neuropsicológicas NBACE, [8,9] grupos de DCL probables o posibles, PDD, PDM y alelo APOE-ε4 (presencia o ausencia de al menos un alelo *APOE*-ε4).

Con el fin de informar de los tiempos de supervivencia estimados se empleó el estimador de Kaplan–Meier, un estimador no paramétrico de la función de supervivencia. La heterogeneidad se calculó para el análisis de datos epidemiológicos.

Por otra parte, al analizar la relación entre *APOE*-ε4 y las variables de la batería NBACE, [8,9] se realizó un Análisis de Regresión Estratificado por alelo *APOE*-ε4. Por último, se llevó a cabo un Análisis de Supervivencia Multivariante sin ajustar por sexo, edad y escolaridad para informar de los efectos acumulativos de las medidas. Todas las hipótesis se probaron bidireccionalmente con un nivel de confianza del 95%.

Estudios prospectivos: Técnicas estadísticas y herramientas bioinformáticas para el procesado de la información generada.

1. **Estudio con biomarcadores de neuroimagen:** se analizaron el volumen de hipocampo ajustado (VHa) de la RM estructural, y las medidas del ratio del valor de captación estandarizado (SUVR) del PET-FDG y del PET-PIB. Se calcularon las correlaciones parciales ajustadas por edad, género y escolaridad con el valor de significación asociado entre cada una de las puntuaciones de los cinco *Composites* Cognitivos y los tres biomarcadores de neuroimagen. Los resultados se replicaron en una muestra de sujetos diagnosticados de DCL debido a EA con afectación de la memoria a nivel de almacenamiento de la base de datos "Alzheimer's disease Neuroimaging Initiative" (ADNI).

2. **Correlación fenotipo-genotipo:** Se midió el efecto de los marcadores genéticos de interés, tipo SNPs, o combinaciones de SNPs usando puntuaciones combinadas de genotipos (PGS) y los factores demográficos mediante análisis de supervivencia. Se realizaron análisis univariantes usando modelos de riesgos proporcionales de Cox Para los 40 SNPs de forma individual (41 incluyendo *APOE*) y además multivariantes para los tres PGS (sin incluir *APOE*) que se calcularon acorde al método descrito por Purcell et al. [146] y que se construyeron con los SNPs incluidos basados en el amplio meta-GWAS publicado por el consorcio IGAP. [100] Los loci seleccionados fueron*: CLU, PICALM, CR1, BIN1, EXOC3L2, ATP5H/KCTD2, ABCA7, MS4A6A, MS4A4E, CD2AP, EPHA1, y CD33* y los once nuevos loci adicionales *HLA-DRB5-HLA-DRB1, PTK2B, SORL1, SLC24A4-RIN3, INPP5D, MEF2C, NME8, ZCWPW1, CELF1, FERMT2 y CASS4*. [100] La PGS1 comprendió nueve loci de riesgo de EA publicados con anterioridad al estudio realizado por el consorcio IGAP [100] (*CR1, BIN1, CD2AP, EPHA1, CLU, MS4A4A, PICALM, ABCA7 y CD33*). La PGS 2 comprendió nueve de los once nuevos loci de riesgo encontrados por IGAP [100] debido a problemas técnicos con ciertos marcadores como rs9271192 en *HLA-DRB5-HLA-DRB1* y para rs10838725 en *CELF1*. La PGS3 se construyó incluyendo los dieciocho loci anteriores (PGS1 +PGS2).

3. **Validación independiente de los resultados:** Se realizó el cotejado cruzado de todas las señales obtenidas en las cuatro series empleando exactamente los mismos modelos estadísticos.

4. **Meta-análisis:** Se realizaron los subsiguientes meta-análisis sistemáticos para la

estimación de los efectos globales tanto de los SNPs como de los tres PGS en las cuatro series independientes, AgeCoDe, DCN, ACE y ADC mediante el enfoque estándar del efecto fijo implementado en el software YAMAS. [115]

5. **Asociación endofenotipo-genotipo:** Las asociaciones entre endofenotipos neuropsicológicos de la batería NBACE [8,9] y los marcadores genéticos de interés, se midieron a nivel basal a través de un análisis de regresión lineal multivariante utilizando el software PLINK (versión 1.07) en la muestra ampliada de sujetos con DCL de Fundació ACE (n=1.250). Las variaciones genéticas se estudiaron, adicionalmente, calculando la frecuencia alélica en nuestra población, cálculo del *PIC* (*polymorphism information content*), equilibrio de Hardy-Weinberg, desviaciones de la frecuencia alélica entre los grupos de estudio empleando test Chi-cuadrado o test exacto de Fisher (cuando fue necesario), LD con marcadores flanqueantes. La representación gráfica de los valores de significación en la asociación entre endofenotipos neuropsicológicos y SNPs se llevó a cabo mediante Mapas de calor (*Heat Maps*) y utilizando el software informático para el análisis estadístico *R* (versión 3.3.1).

6. Aspectos éticos

1. Colección de muestras biológicas disponible.

La Colección de Fundació ACE – C.0000299, está registrada en el Registro Nacional de Biobancos. La Fundació ACE lleva recogiendo material biológico de sus pacientes

desde hace más de quince años. El protocolo ético actual, aprobado por el CEIC del Hospital Clínic de Barcelona y de acuerdo con la Ley Española de Biomedicina, ha permitido reclutar muestras sanguíneas de más de 12000 sujetos atendidos en la unidad de memoria. El proyecto de reclutamiento engloba sujetos con cualquier tipo de demencia, personas con DCL, sujetos con quejas subjetivas de memoria y controles sanos. Los individuos otorgan un consentimiento informado amplio y multinivel que permite el uso del material para la ejecución de nuevos proyectos de investigación sin necesidad de re-consentir los especímenes siempre y cuando el comité de ética apruebe el nuevo proyecto. Asimismo, el estudio con biomarcadores de neuroimagen, fue aprobado por el comité ético del CEIC 2009/5455, y todos los participantes aportaron su consentimiento informado por escrito previamente a la inclusión en el estudio.

2. Generación de la base de datos de pacientes, biobanco y extracción del ADN de los pacientes en estudio.

El proceso de inclusión de pacientes y de recogida de muestras y datos clínicos constó de las siguientes etapas:

a. Información al paciente acerca de las características del proyecto, riesgos y beneficios.

El neurólogo informó al paciente del proyecto, de sus riesgos y de los beneficios que se esperan obtener, entregando una hoja con toda la información.

b. Obtención del consentimiento informado por escrito.

Este consentimiento, firmado por el paciente contuvo el código de barras que se asignó al paciente en el estudio y sus datos personales. Este documento fue retenido y archivado en el centro de investigación clínica y será destruido una vez concluido el estudio de acuerdo con la legislación vigente.

c. Extracción de muestra biológica.

Una vez que el paciente otorgó su consentimiento informado, se obtuvieron 15 ml de sangre periférica, mediante venopunción. Esta intervención la realizó personal de enfermería cualificado de Fundació ACE. Las muestras sanguíneas de los pacientes se acumularon a –20º C hasta un máximo de 30 días. Se efectuaron envíos periódicos del material sanguíneo al laboratorio para su procesado.

d. Obtención de ADN.

Las muestras sanguíneas obtenidas fueron procesadas en los laboratorios para la obtención de ADN de alto peso molecular empleando la tecnologías MagnaPure (Roche) o Maxwell 16 (Promega) y fueron criopreservadas a –20º C. Los datos clínicos adscritos de cada muestra fueron procesados y organizados en bases de datos relacionales para su utilización en el rastreo de genes de la patología en estudio.

Las características de las muestras, las pruebas neuropsicológicas, genéticas y de neuroimagen, así como los análisis estadísticos utilizados se encuentran descritos detalladamente en cada uno de los cuatro estudios correspondientes.

6. RESULTADOS

Artículo I

6.1. A longitudinal follow-up of 550 Mild Cognitive Impairment patients: evidence for large conversion to dementia rates and detection of major risk factors involved.

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A Longitudinal Follow-Up of 550 Mild Cognitive Impairment Patients: Evidence for Large Conversion to Dementia Rates and Detection of Major Risk Factors Involved

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Abstract. The most recent studies about mild cognitive impairment (MCI) are focused on the search for factors that make patients more vulnerable to conversion to dementia, mainly Alzheimer's disease (AD). The aim of this study was to determine which neuropsychological test performances, including episodic memory profiles, and genetic risk factors (*APOE* ε4) better predict early conversion to dementia among the four MCI subtypes. Data from 550 MCI patients were analyzed for the purpose of this study and were classified according to Petersen's criteria (2004), and also taking into account the absence (probable MCI) or presence (possible MCI) of comorbidities that could explain cognitive deficits. MCI cases were divided into Probable amnestic (Pr-aMCI) ($n = 115$), probable non-amnestic (Pr-naMCI) ($n = 37$), possible amnestic (Pss-aMCI) ($n = 234$), and possible non-amnestic (Pss-naMCI) ($n = 164$), single or multiple domain. In the whole MCI sample, regression analysis showed that low performances on Orientation, Verbal Delayed Recall of the Word List Learning test from WMS-III, and Luria's Clock test were associated with conversion to dementia, independently of *APOE* ε4 allele. Cox proportional-hazards showed that the Probable MCI subtype, presence of storage memory impairment, multiple domain condition, and presence of at least one ε4 allele increased the risk of conversion to dementia. Multivariate survival and Kapplan-Meier analyses showed that the Pr-aMCI with storage memory impairment had the most and closest risk of conversion to dementia. In conclusion, the Pr-aMCI subset of patients had 8.5 times more risk of converting to dementia than the Pss-naMCI group, who displayed the slowest conversion rate to dementia.

Keywords: Amnestic, cognition, dementia conversion, genetics, mild cognitive impairment, risk factors

Supplementary data available online: http://www.j-alz.com/issues/34/vol34-3.html#supplementarydata03

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INTRODUCTION

Mild cognitive impairment (MCI) is a clinically heterogeneous syndrome. Its first classification was based on memory impairment, but it was later expanded to other cognitive domains. According to Petersen et al. [1] classification, MCI would comprise four broad subgroups depending on memory performance and the number of impaired cognitive functions: amnestic single (aMCI-sd) and multiple domains (aMCI-md), and non-amnestic single (naMCI-sd) and multiple domains (naMCI-md). Other classification schemes have taken into account the presence (possible MCI) or absence (probable MCI) of comorbidities (i.e., cerebrovascular disease, history of head trauma encephalopathy, infectious diseases, or developmental disabilities) that could explain observed cognitive deficits [2, 3].

Over the last decades, most research on MCI [4] has been focused on searching for factors which make patients more vulnerable to conversion to dementia, in particular Alzheimer's disease (AD). Recently, the International Working Group for New Research Criteria for the Diagnosis of AD introduced the definition of prodromal AD to describe a symptomatic disease phase or the predementia stage of AD [5].

The annual conversion rate of amnestic MCI to AD reported in different studies rises from 10–19% [6–8], 30% [9, 10], to 40% [11]. Some recent studies suggested that the risk of AD increases when additional domains besides memory are impaired, probably because they are in a more advanced stage of the neurodegenerative disease [12–16].

In contrast, other studies have found lower conversion rates to AD in aMCI-md and naMCI, than in those with aMCI-sd, suggesting that neuropsychological variables other than memory are not useful to predict progression to AD [9, 17].

Although it is well known that memory is one of the main risk factors to dementia conversion in MCI [5–7, 18–21], one of the most frequent limitations of several studies consists of the lack of an exhaustive assessment of memory functioning and the scarce sample size analyzed. In some cases, a recognition memory test in the memory assessment was missing [22, 23], impeding a comprehensive detection of which parameters of episodic memory (failures in encoding, storage, retrieval, or recognition) are the most vulnerable to prodromal AD and other dementias.

In this study we report a longitudinal follow-up of 550 MCI individuals, representing one of the largest single-site clinical MCI series reported worldwide. Routine clinical and neuropsychological follow-up of people with MCI allowed us to comprehensively estimate conversion rates in different MCI subtypes and to determine neuropsychological test performances, including the episodic memory profiles that predict early conversion to dementia in different MCI subtypes.

METHODS

Subjects

For the purpose of this study, data was analyzed from a sample of 550 individuals who visited the Diagnostic Unit of Fundació ACE (Barcelona, Spain) between January 2006 and November 2011 and were diagnosed as MCI. These patients had the following characteristics: a Clinical Dementia Rating Scale (CDR) [24] of 0.5, older than 60 years of age, functionally literate, no severe auditory or visual abnormalities including glaucoma and cataracts, and available DNA sample. Their medical records were reviewed to classify them according to Petersen's criteria [1, 6] (amnestic and non-amnestic, single and multiple domains) and Lopez et al. [2] classification. Possible MCI was assigned only when vascular or psychiatric etiology was suspected.

Diagnostic adjudication

All subjects were recruited and assessed at the Fundació ACE and all diagnoses were assigned at a consensus conference among neurologists, neuropsychologists, and social workers. All subjects had at least one follow-up (mean follow-up time: 26.6 months; range: 6–68 months) and were older than 60, and all follow-up diagnoses were made with full knowledge of prior classification and prior neurobehavioral data. At time of enrollment, all patients fulfilled MCI Petersen's diagnostic criteria [1, 6], including subjective memory complaints, normal general cognition, preserved performance in activities of daily living, absence of dementia, and a measurable impairment in one or more cognitive functions. All MCI subjects had a CDR rating of 0.5 and none were taking any dementia medication (i.e., acetylcholinesterase inhibitors or memantine).

Domain Pattern Impairment (DPI): Amnestic and non-amnestic single and multiple domain MCI criteria

MCI was subclassified as aMCI-sd, aMCI-md, naMCI-sd, and naMCI-md fulfilling Petersen's criteria [1, 6], including subjective memory complaints,

normal general cognition, preserved performance in activities of daily living, absence of dementia, and a measurable impairment in memory function, with or without deficit in other cognitive domains (amnestic MCI single domain or amnestic MCI multiple domain).

Memory Pattern Impairment (MPI): Storage and retrieval amnestic MCI criteria

The amnestic MCI patients (aMCI-sd and aMCI-md) with impaired delayed verbal recall for whom recognition testing did not improve performance were classified as having an "Encoding/Storage" pattern of memory loss. In contrast, patients with impaired verbal delayed recall, but for whom testing using a recognition format resulted in greatly improved performance were classified as having a "Retrieval" deficit.

Possible and probable MCI criteria

Similar to Lopez et al. [2] classification, but extending it to the non-amnestic MCI groups (naMCI-sd and naMCI-md), all MCI subjects were reclassified as possible MCI when there were comorbidities that could explain or contribute to cognitive deficits; and they were classified as probable MCI when there were none. Therefore, subjects were classified as having Pss-aMCI when there were psychiatric, neurological (i.e., cerebrovascular disease, history of head trauma encephalopathy, infectious diseases, or developmental disabilities), or systemic illnesses that could cause cognitive deficits or when there was insufficient information. In the present study, for the Pss-aMCI and Pss-naMCI groups, only those subjects with cerebrovascular disease and psychiatric disorders (anxiety or depression) were included. In contrast, subjects were classified as having Pr-aMCI or Pr-naMCI if there were no neurological, psychiatric, or systemic illnesses that could explain their cognitive deficits.

Converters and non-converters MCI criteria

Subjects who converted to dementia, that is AD [25, 26], vascular dementia [27], mixed dementia (AD with cerebrovascular disease), frontotemporal dementia [28, 29], or dementia with Lewy bodies [30] over the study period, were classified as MCI converters. All of these subjects had a CDR [24] of 1. In contrast, those subjects who remained stable during follow-ups were classified as Stable or non-MCI converters. Finally, patients who normalized performance, with or without subjective memory complaints ($n = 10$), were excluded from this study.

APOE genotyping

The *APOE* ε4 allele was identified with commercial kits for *APOE* rs429358 (SNP112) and rs7412 (SNP158) from Roche Diagnostics (Germany). The *APOE* alleles were amplified using LightCycler ApoE Mutation Detection Kit (Roche diagnostics, Germany) and were detected using real-time PCR technology (LightcyclerR 480 System, Roche Diagnostics, Germany) following the manufacturer's instructions. To check the quality of the results, different compound heterozygotes for *APOE* SNPs were verified in an independent research laboratory. Only one blood sample was not genotyped due to it being accidentally damaged.

Clinical assessment

All participants received standardized neurobehavioral exams, including neurological examination, neuropsychological testing, and social work evaluations.

Information about vascular risk factors (including hypertension, hypercholesterolemia, diabetes mellitus, history of stroke, heart disease, and family history of dementia) was provided by the patients or their caregivers. All subjects were examined with the Mini-Mental State Examination [31], Hachinski Ischemia Scale [32], and CDR [24]. The MRI scans, or more usually CT scans, were available for review. A comprehensive neuropsychological protocol was administered to all subjects. MCI with vascular disease were identified according to an algorithm previously described in detail [33]. Briefly, several expressions of significant vascular disease (e.g., hypertension, angina pectoris) and findings on MRI (or CT), associated with vascular disease were required for a classification of possible MCI of vascular etiology.

Neuropsychological assessment

The neuropsychological battery of Fundació ACE (NBACE) [34] was administered to all MCI patients. This diagnostic procedure included tests sensitive to processing speed, orientation, attention, verbal learning and memory, language, visuoperception, gnosis, praxis, and executive functions, including the following tests: Temporal, Spatial and Personal Orientation; Digit spans (forwards and backwards), Block Design

(abbreviated so that items 6 to 9 were scored only for accuracy (1 point) without a time bonus) and Similarities (abbreviated to the first 10 items) subtests of Wechsler Adult Intelligence Scale-Third Edition (WAIS-III); The Word List Learning test from the Wechsler Memory Scale-Third Edition (WMS-III) (without using the interference list); Repetition (2 words and 2 sentences); Verbal comprehension (to correctly execute 2 simple, 2 semi-complex and 2 complex commands extracted from the ADAS-cog and the Barcelona test battery; the abbreviated 15-item Boston Naming Test; the Poppelreuter test; Luria's Clock test; The 15-Objects test; the Automatic Inhibition subtest of the *Syndrom Kurtz Test* (SKT); Phonetic Verbal Fluency (words beginning with 'P' in one minute); Semantic Verbal Fluency ('animals' in one minute); and the Spanish version of the Clock Test.

Statistical analysis

Statistical analysis was performed using commercially available software (SPSS for Windows, v18.0, SPSS Inc, Chicago, IL). To compare demographic, clinical, and genetic data among the four groups, an Analysis of Variance (ANOVA) or a Chi square were carried out for the quantitative and qualitative variables, respectively. To compare neuropsychological performances on the NBACE tests at baseline an Analysis of Covariance (ANCOVA), adjustments for gender, age, and education level were made. When a variable was statistically significant, Bonferroni *post-hoc* multiple comparisons were performed. To analyze predictors of conversion to dementia, Cox proportional hazards, adjustments for gender, age, and education were made introducing different variables according to the different models considered: NBACE variables, probable or possible MCI criteria, DPI, MPI, and *APOE* ε4 allele (presence or absence of at least one *APOE* ε4 allele). Kapplan-Meier survival analysis was also executed in order to report estimated survival times. Heterogeneity was computed using Episheet by Ken Rothman (available at: http://www.drugepi.org/wp-content/uploads/2012/10/Episheet.xls). Moreover, to analyze the relationship between *APOE* ε4 and NBACE variables, a regression analysis stratified by *APOE* ε4 allele was performed. Finally, a multivariate survival analysis without adjusting for gender, age, and education was carried out to report cumulative effects of the measures. All hypotheses were tested bidirectionally at a 95% confidence level.

RESULTS

Data from the 550 MCI individuals of this study were classified in the following MCI subtypes: Probable amnestic MCI subjects (Pr-aMCI) ($n = 115$; 20.9%), possible amnestic MCI subjects (Pss-aMCI) ($n = 234$; 42.5%), probable non-amnestic MCI individuals (Pr-naMCI) ($n = 37$; 6.7%), and possible non-amnestic MCI individuals (Pss-naMCI) ($n = 164$; 29.8%). In the whole sample, most of MCI patients displayed a multiple domain impairment ($n = 451$; 88%) whereas a single domain affection appeared only in 99 subjects ($n = 99$; 12%). Regarding amnestic MCI individuals, a storage pattern of memory impairment was observed in 299 subjects (65%) and a retrieval impairment was found in 120 subjects (35%).

Demographic, clinical, and genetic data of patients are detailed in Table 1. The prevalence of subjects in MCI subtypes was as follows: 20.9% Pr-aMCI, 42.5% Pss-aMCI, 6.7 % Pr-naMCI, and 29.8% Pss-naMCI. Average follow-up time for all the subjects was 26.6 months (SD: 15.5, range: 6–68). Although no statistically significant differences among MCI groups were found in age and gender, they did significantly differ in educational level (see Table 1). All the analyses were adjusted by age, gender, and education.

According to the DPI classification criteria [4], most of MCI patients displayed a multiple domain cognitive impairment, and multiple domain was more common in amnestic compared to non-amnestic MCI ($\chi^2 = 553.90$; $p = 0.001$: Pr-aMCI-md: 86.1%, Pss-aMCI-md: 88.5%, Pr-naMCI-md: 64.9%, and Pss-naMCI-md: 73.8%).

In the amnestic MCI groups, in terms of the MPI, classified as a "Storage" or "Retrieval" pattern of memory loss, a memory storage deficit was more frequent ($\chi^2 = 581.14$; $p = 0.001$) among those subjects with Pr-aMCI (81.7%) than in Pss-aMCI patients (57.7%).

Regarding *APOE* genotype, the comparison of *APOE* ε4 genotyping (presence or absence of at least one ε4 allele) among MCI groups showed a higher frequency of ε4 allele in Pr-aMCI than in Pss-aMCI, Pr-naMCI, and Pss-naMCI groups. There was no subject with the ε2 alleles (see Table 1). The Pr-aMCI had a higher frequency of ε4 allele than the rest of subjects (that is, Pss-aMCI, Pr-naMCI, and Pss-naMCI groups together) ($\chi^2 = 32.53$; $p < 0.0001$).

With regard to the clinical variables, such as hypercholesterolemia, hypertension, diabetes, heart disease, smoking habit, alcohol abuse, stroke, and family history of dementia, none of them showed statistically significant differences among

Table 1
Comparison of demographic, clinical, and genetic data between groups

	Pr-aMCI	Pss-aMCI	Pr-naMCI	Pss-naMCI	Statistics	*p*
n (%)	115 (20.9)	234 (42.5)	37 (6.7)	164 (29.8)		
Gender, *n* (%) Female	73 (63.5)	154 (65.8)	21 (56.8)	122 (74.4)	6.61[1]	0.085
Education in years, *n* (%)					25.19[1]	0.001
<6	47 (40.9)	137 (58.5)	11 (29.7)	100 (61.0)		
6–11	51 (44.3)	80 (34.2)	20 (54.1)	55 (33.5)		
>11	17 (14.8)	17 (7.3)	6 (16.2)	9 (5.5)		
Age in years (mean/SD)	76.5/6.1	76.3/6.4	76.1/5.2	75.7/7.0	0.45[2]	0.715
MMSE (mean/SD)	25.3/2.7	25.1/3.0	27.0/2.0	26.8/2.5	16.40[2]	0.001
HIS (mean/SD)	1.7/1.1	2.4/1.8	2.2/1.9	2.5/1.6	5.38[2]	0.001
APOE 4 (ε4 or ε4/ε4), n (%)	62 (53.9)	63 (26.9)	6 (16.2)	44 (27.0)	34.33[1]	0.001
ε2/ε3 n (%)	4 (3.5)	11 (4.7)	3 (8.1)	12 (7.3)		
ε2/ε4 n (%)	4 (3.5)	8 (3.4)	0	2 (1.2)		
ε3/ε3 n (%)	49 (42.6)	160 (68.4)	28 (75.7)	107 (65.2)		
ε3/ε4 n (%)	47 (40.9)	49 (20.9)	6 (16.2)	39 (23.8)		
ε4/ε4 n (%)	11 (9.6)	6 (2.6)	0	3 (1.8)		
Hypercholesterolemia	45/114	104/225	17/36	81/160	3.35[1]	0.341
Hypertension	68/114	137/227	23/37	91/160	0.63[1]	0.891
Diabetes	20/113	43/227	4/36	24/158	1.90[1]	0.595
Heart disease	28/113	45/226	9/36	27/156	2.73[1]	0.434
Smoking habit	6/114	11/229	2/36	12/157	1.46[1]	0.691
Alcohol	2/113	9/226	3/36	9/158	3.97[1]	0.265
Family history of dementia	51/112	93/226	17/36	56/158	3.54[1]	0.315

Pr-aMCI, probable amnestic mild cognitive impairment; Pss-aMCI, possible amnestic mild cognitive impairment; Pr-naMCI, probable non-amnestic mild cognitive impairment; Pss-naMCI, possible non-amnestic mild cognitive impairment; MMSE, Mini-Mental State Examination; HIS, Hachinski Ischemia Scale; SD, standard deviation; [1] χ^2; [2] F.

the four MCI groups. However, Pr-aMCI and Pr-naMCI subjects obtained lower scores on Hachinski Ischemia Scale than Pss-aMCI and Pss-naMCI groups (Table 1).

The ANCOVA showed statistically significant differences among groups in several neuropsychological variables (Table 2). Ideomotor praxis, repetition, and verbal comprehension variables were not introduced in the analysis because they were practically a constant in all groups. However, Bonferroni *post-hoc* multiple comparisons analyses revealed that only performance on Verbal Delayed Recall and Recognition memory subtests of the WMS-III showed statistically significant differences among the four MCI groups. Moreover, in order to report effect of presence or absence of at least one *APOE* ε4 allele at baseline, on neuropsychological performance, mean comparisons were executed and only Constructional Praxis ($p = 0.02$) and Delayed recall ($p = 0.004$) showed differences among four groups.

Follow-up analysis of MCI patients (mean follow-up time: 26.6 months; range: 6–68 months) indicated that Pr-aMCI converted to dementia in a higher proportion (30.4% Stable versus 69.6% Converters) than Pss-aMCI (47.4% Stable versus 52.6% Converters), followed by Pr-naMCI (54.1% Stable versus 45.9% Converters), and Pss-naMCI (77.4% Stable versus 22.6% Converters), respectively ($\chi^2 = 65.78$; $p = 0.001$).

Survival time of conversion to dementia differed substantially among MCI subtypes. Patients with Pr-aMCI were the nearest to dementia conversion. Median time for the Pr-aMCI was 21 months (15.91–26.09 [95% CI]), while for the Pss-aMCI it was 34 months (28.78–39.22 [95% CI]) (Wald = 11.91; $p = 0.001$; OR = 2.658), followed by Pr-naMCI with a mean time of 42 months (33.77–50.23 [95% CI]) (Wald = 61.87; $p = 0.001$; OR = 4.717), and finally for the Pss-naMCI, it was 62 months (52.30–71.72 [95% CI]) (Wald = 32.59; $p = 0.001$; OR = 2.870) (Fig. 1).

A total of 257 (46.7%) subjects developed dementia during follow-up. Of these, 117 (45.5%) developed AD, 51 (19.8%) vascular dementia, 50 (19.5%) mixed dementia, 25 (9.7%) frontotemporal dementia, 12 (4.7%) dementia with Lewy bodies, and 2 (8%) Parkinson dementia. In the analyses below, we first report factors associated with a risk to develop any dementia syndrome during follow-up, concerning the whole sample, followed by the analysis for each of the four MCI subtypes.

In terms of the single or multiple MCI classification (DPI), in the whole sample, median survival time was 36 months (19.98–52.02 [95% CI]) for the single domain MCI, and 27 months

Table 2
The ANCOVA comparing neuropsychological scores between MCI groups

							Multiple comparisons with Bonferroni's correction		
							Pr-aMCI versus		
	Pr-aMCI M (SD)	Pss-aMCI M (SD)	Pr-naMCI M (SD)	Pss-naMCI M (SD)	F (3, 537)	Eta^2	Pss-aMCI	Pr-naMCI	Pss-naMCI
Global orientation	13.51 (1.67)	13.90 (1.41)	14.15 (1.22)	14.47 (1.03)	12.05***	0.06	NS	NS	0.001**
Verbal learning and memory WMS-III									
Learning (Trials 1+2+3+4)	15.79 (5.11)	17.19 (4.80)	22.63 (5.80)	22.61 (4.88)	72.54***	0.29	NS	0.001***	0.001***
Delayed recall	0.78 (1.03)	1.40 (1.34)	4.63 (1.92)	5.22 (1.78)	338.82***	0.65	0.001***	0.001***	0.001***
Recognition memory	17.76 (2.77)	18.82 (2.72)	21.43 (1.63)	21.66 (1.86)	75.79***	0.30	0.001***	0.001***	0.001***
Attention and working memory									
Forward digits	6.61 (1.66)	6.48 (1.74)	6.84 (1.83)	6.60 (1.45)	0.68	0.00	NS	NS	NS
Backward digits	3.49 (1.50)	3.13 (1.46)	3.62 (2.04)	3.53 (1.45)	4.92**	0.03	NS	NS	NS
Praxis									
Block design	3.04 (1.06)	3.02 (1.07)	3.32 (0.87)	3.06 (1.10)	0.92	0.01	NS	NS	NS
Imitation	3.05 (1.02)	3.03 (1.05)	3.35 (0.97)	3.37 (0.87)	4.73**	0.03	NS	NS	NS
Language									
Visual naming (15-BNT)	12.68 (2.41)	12.64 (2.39)	13.25 (1.50)	13.32 (1.87)	4.00*	0.02	NS	NS	NS
Visual perception									
Poppelreuter test (responses)	8.86 (1.43)	8.90 (1.36)	9.32 (0.80)	9.22 (1.15)	3.47*	0.02	NS	NS	NS
Luria's Clocks test	2.67 (1.16)	2.63 (1.21)	3.04 (1.13)	2.73 (1.13)	1.48	0.01	NS	NS	NS
The 15-Objects test (responses)	11.45 (2.10)	11.19 (1.65)	12.76 (.58)	12.44 (1.81)	1.52	0.09	NS	NS	NS
Executive functions									
SKT (time in seconds)	41.82 (16.82)	45.75 (26.54)	40.13 (12.85)	41.13 (13.89)	2.32	0.08	NS	NS	NS
SKT (errors)	4.29 (5.19)	4.63 (5.28)	3.67 (3.33)	3.24 (4.55)	2.75*	0.02	NS	NS	NS
PVF	9.13 (4.09)	7.83 (4.07)	9.90 (4.28)	9.19 (3.94)	6.60***	0.04	0.011*	NS	NS
SVF	11.22 (3.69)	11.02 (3.55)	13.17 (4.51)	12.91 (3.57)	11.85***	0.06	NS	0.024*	0.001***
Similarities WAIS-III	7.66 (3.13)	7.80 (3.09)	8.84 (2.53)	8.48 (2.60)	3.87*	0.02	NS	NS	NS
Global Cognition Clock Test	4.82 (2.22)	4.91 (2.07)	5.95 (1.60)	5.64 (1.72)	8.17***	0.05	NS	NS	0.001***

Global orientation, summary of Temporal + Spatial + Personal orientations; WMS-III, Wechsler Memory Scale, Third Edition; Abbreviated BNT, Boston Naming Test with 15 visual items; Recognition memory, correct answers; Block Design, WAIS-III; SKT, Automatic Inhibition Symdrom Kurztest; PVF, phonemic verbal fluency; SVF, semantic verbal fluency; WAIS-III, Weschler Adult Intelligence Scale, Third edition; M, mean; SD, standard deviation; NS, $p \geq 0.05$; *$p < 0.05$, **$p \leq 0.005$, ***$p \leq 0.001$.

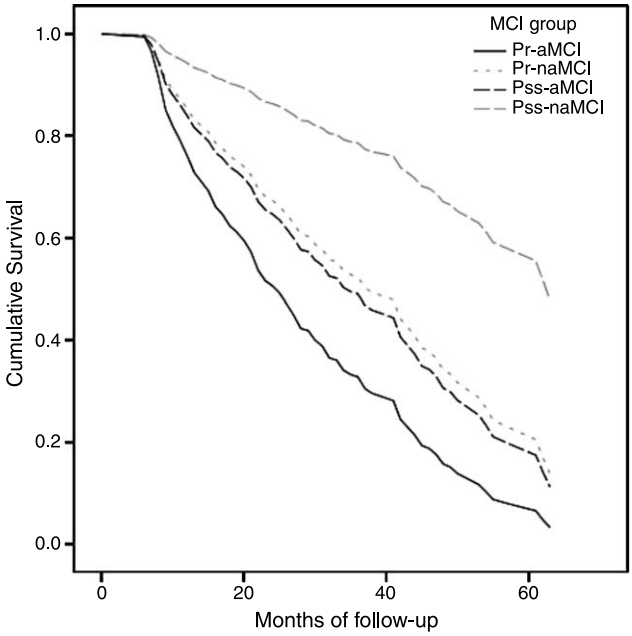

Fig. 1. Survival time of conversion to dementia. Non-converters MCI ($n = 293$) were censored (53.3%).

Table 3
MCI subtype, APOE ε4, and conversion to dementia

		AD	VaD	MD	DLB	FTD	PD
Pr-aMCI ($n = 89$)							
n (%)	ε4 (%)	58 (52.5)	6 (7.5)	8 (10.0)	3 (3.8)	5 (6.3)	0 (0.0)
APOE ε4	Present	35 (76.1)	3 (6.5)	4 (8.7)	1 (2.2)	3 (6.5)	0 (0.0)
Status	Absent	23 (67.6)	3 (8.8)	4 (11.8)	2 (5.9)	2 (5.9)	0 (0.0)
Pss-aMCI ($n = 125$)							
n (%)	ε4 (%)	39 (31.7)	38 (30.9)	30 (24.0)	5 (4.1)	10 (8.1)	2 (1.6)
APOE ε4	Present	16 (43.2)	10 (27.0)	6 (16.2)	1 (2.7)	4 (10.8)	0 (0.0)
Status	Absent	23 (26.7)	28 (32.6)	29 (23.6)	4 (4.7)	6 (7.0)	2 (2.3)
Pr-naMCI ($n = 20$)							
n (%)	ε4 (%)	8 (47.1)	0 (0.0)	2 (13.3)	2 (11.8)	5 (29.4)	0 (0.0)
APOE ε4	Present	2 (100)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Status	Absent	6 (40.0)	0 (0.0)	2 (11.8)	2 (13.3)	5 (33.3)	0 (0.0)
Pss-naMCI ($n = 39$)							
n (%)	ε4 (%)	12 (32.4)	7 (18.9)	11 (29.7)	2 (8.3)	5 (13.5)	0 (0.0)
APOE ε4	Present	6 (46.2)	3 (23.1)	4 (30.8)	0 (0.0)	0 (0.0)	0 (0.0)
Status	Absent	6 (25.0)	4 (16.7)	7 (29.2)	2 (5.4)	5 (20.8)	0 (0.0)

AD, Alzheimer's disease; VaD, vascular dementia, MD, mixed dementia; DLB, dementia with Lewy bodies; FTD, frontotemporal dementia; PD, Parkinson's disease.

(23.18–30.82 [95% CI]) for multiple domain MCI (*Wald* = 3.23; $p = 0.072$; OR = 1.56). For the four MCI subtypes (see Supplementary Table 1; available online: http://www.j-alz.com/issues/34/vol34-3.html#supplementarydata03), there were only significant differences for Pr-aMCI with a median survival time of 38 months (19.98–56.02 [95% CI]) for the single domain MCI, and 20 months (15.13–24.87 [95% CI]) for multiple domain MCI (*Wald* = 3.62; $p = 0.057$; OR = 2.09). In contrast, no statistically significant differences were found among single and multiple domains MCI in any other group, that is, it was not significant for Pss-aMCI, for Pr-naMCI, or for Pss-naMCI.

Heterogeneity analysis for DPI (Supplementary Table 1) in the four MCI subtypes, suggested that DPI ($p = 0.008$) showed heterogeneous effects among the four MCI subtypes.

Regarding the storage or retrieval MPI for the whole aMCI group, median survival time was 27 months

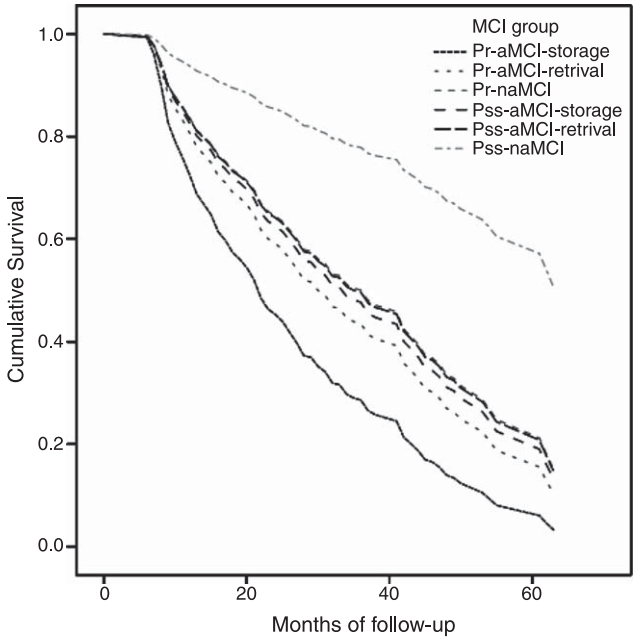

Fig. 2. Multivariate survival analysis. Non-converters MCI ($n = 293$) were censored (53.3%).

(22.33–31.67 [95% CI]) for the storage group, and 31 months (24.54–37.46 [95% CI]) for the retrieval MPI group (*Wald* = 3.55; $p = 0.059$; OR = 1.34). However, for the amnestic MCI subtypes (Supplementary Table 1), there were not statistically significant differences on survival time among storage and retrieval groups, that is, it was not significant for Pr-aMCI, neither for Pss-aMCI, in heterogeneity analysis ($p = 0.393$).

With regard to *APOE*, the presence of at least one ε4 allele did significantly increase conversion to dementia in the whole MCI group, with a survival median time of 31 months (23.76–38.24 [95% CI]) for carriers of ε4 and 44 months (38.43–49.57 [95% CI]) for non-carriers (*Wald* = 19.89; $p = 0.001$; OR = 1.80). In the analysis for each of the four MCI subtypes (see Supplementary Table 1), only the Pss-aMCI group showed statistically significant differences for carriers of ε4. No statistically significant differences were found in the Pss-naMCI, Pr-aMCI, or Pr-naMCI groups. However, heterogeneity analysis for *APOE* ε4 ($p = 0.330$) (Supplementary Table 1) in the four MCI found no heterogeneous effects among the four MCI subtypes. It must be mentioned that for all MCI subtypes the majority of subjects converted to AD (see Table 3).

The neuropsychological variables that predicted conversion to dementia for the whole MCI group were the following: Orientation (0.76–0.91 [95% CI]), (Wald = 15.14; $p = 0.001$; OR = 0.83); Verbal Delayed Recall on WMS-III (0.73–0.86 [95% CI]), (Wald = 29.51; $p = 0.001$; OR = 0.79); and Luria's Clock test (0.71–0.90 [95% CI]), (Wald = 13.19; $p = 0.001$; OR = 0.80). For each of the four MCI subtypes (Supplementary Table 1), the following neuropsychological predictors of conversion to dementia were significant: Pr-aMCI: Orientation, Learning, Verbal Delayed Recall, and Recognition memory; Pss-aMCI: Orientation, Verbal Delayed Recall, Luria's Clocks test, and Semantic verbal fluency; Pr-naMCI: Orientation and Recognition memory; Pss-naMCI: Orientation, Verbal Delayed Recall, and Luria's Clocks test. Heterogeneity analysis for NBACE tests (Supplementary Table 1) in the four MCI subtypes, suggested that only Orientation ($p = 0.032$) and Recognition ($p = 0.017$) showed heterogeneous effects among the four MCI subtypes.

Moreover, in the four MCI subtypes, the regression analysis stratified by *APOE* ε4 allele showed that performance on Orientation, Verbal Delayed Recall, and Luria's Clocks test were independent of the presence of *APOE* ε4 allele.

Finally, a multivariate survival analysis without adjusting for gender, age, and education, was carried out combining the possible-probable, amnestic-non amnestic, and storage-retrieval groups. The results showed that the probable amnestic-storage group had the highest risk of conversion to dementia, having 8.5 times more risk to convert to dementia than the possible

non-amnestic MCI group, the group that resulted to have the slowest conversion to dementia (see Fig. 2).

DISCUSSION

In this study we report a follow-up of 550 classified MCI individuals, representing one of the largest single-site clinical MCI series reported worldwide. Although the classification of MCI into probable or possible subtypes had been previously used, in our knowledge, this is the first study applying it in the non-amnestic group, allowing us to increase the accuracy of the MCI classification.

The main finding of this paper was that the Probable MCI condition had higher risk of early conversion to dementia than the rest of MCI individuals. Moreover, subjects with memory impairment (Pr-aMCI and Pss-aMCI) converted earlier to dementia than the non-amnestic ones. However, except for the Pss-naMCI group, and according to a previous study [3], the prognosis among the groups is very similar.

In accordance with previous reports, the aMCI were found more frequently than the na-MCI type [35–37]. In our study, probable MCI is more frequent than possible MCI subtype. The prevalence for the aMCI (20.9% for Pr-aMCI and 42.5% for Pss-aMCI, respectively); for the naMCI the prevalence was 6.7% for Pr-naMCI and 29.8% for Pss-naMCI. Pr-aMCI displayed a higher risk to develop dementia than Pss-aMCI. Conversely, Pr-naMCI converted more to dementia than the Pss-naMCI group.

According to previous reports [38–40], most patients, irrespective of MCI subtype, who converted to dementia, mainly developed the AD type (45.5%). Although initially it was thought that naMCI had an increased risk of conversion to non-AD dementias [1, 41], in our sample patients with aMCI had more risk of conversion to dementia than naMCI group, but in both cases, mainly to AD.

In terms of DPI, a major distribution of multiple domain patterns was found among all MCI subtypes in our sample, with a major number of Pr-aMCI subjects with multiple domains impaired. Similar results have been reported by other groups [2, 15], but not all [35]. Our data supports that the traditional amnestic single-domain aMCI (aMCI-sd) [6] is rarely diagnosed when a comprehensive neuropsychological battery is applied, because other cognitive impairments are frequently found when neuropsychological evaluation is expanded [43]. More importantly, the naMCI group displaying impairment in several cognitive domains was associated with a faster conversion to AD and dementia compared to naMCI-sd group. This result would suggest that impairment in one cognitive domain alone (other than memory) is a rather benign condition [36]. Furthermore, this result also suggests that memory impairment is not always the first symptom of even the common dementia disorders, and neither is memory impairment specifically associated with an increased rate of progression to dementia [36, 44, 45]. Moreover DPI showed heterogeneous effects, specifically in Pr-aMCI subtype.

In terms of MPI, storage memory deficit was more frequent among Pr-aMCI than in the Pss-aMCI group. It is important to include a recognition memory test in the memory assessment. For obvious reasons, it is impossible to determine a storage memory deficit, without a recognition test. According to other authors [22, 23], a storage deficit is correlated with a higher risk of conversion to dementia for all MCI subtypes compared to a retrieval deficit memory.

Similar to previous genetic studies [46], the distribution of *APOE* alleles in MCI in our series revealed that ε3 was the most frequent (62.5%) and ε2 the least (8.0%) frequent allele. Interestingly, there were no MCI subjects with the ε2/ε2 haplotype, which has been related to protection against late-onset AD [47]. In the present study, the *APOE* ε4 allele was more frequent in Pr-aMCI than in the rest of MCI subtypes. Although the *APOE* ε4 allele was significant after survival analysis only for Pss-aMCI, heterogeneity analysis showed that *APOE* ε4 allele had homogeneous effects in the four MCI subtypes. The presence or absence of at least one ε4 allele affected the conversion rate, supporting the evidence of the degree of clinical heterogeneity that surrounds the MCI syndrome, and suggesting that the ε4 allele may be associated with accelerated neurodegeneration in the development and progression of several neurodegenerative diseases [48, 49]. This also points out the importance of improving diagnosis and follow-up of all the MCI groups. However, *APOE* ε4 is the strongest genetic risk factor for sporadic AD. Thus, it is no surprise that the presence of even a copy of the allele shortens the time to dementia. Regarding the genetic risk characteristics for conversion to dementia in terms of the ε4 allele, according to previous literature, possession of an ε4 allele was associated with an increase of the risk of developing AD [50]. In our sample, for aMCI carriers of ε4, they are about 1.7 times more likely to develop AD than non-carriers, and still, for the naMCI carriers of ε4, they are about 1.2 times more likely to develop AD than non-carriers.

Our results suggest that it is very important to include tests sensitive to delayed recall (including a

recognition memory task), orientation, and visuospatial gnosis in the neuropsychological assessment of all MCI subtypes. For the Pss-naMCI group, our results are in accordance with a previous study [42] where a poor performance on Delayed recall in non-amnestic MCI predicts progression to dementia, overall in those patients with a multiple domain impairment especially in global orientation and visuospatial gnosis (Luria's clock tests). Our study demonstrated that Orientation and verbal recognition memory are of great importance in the assessment of the amnestic and non-amnestic probable MCI subtypes. This is important because memory might be preserved in na-MCI, but a poor performance in the Recognition task in the Pr-naMCI constitutes a risk factor for conversion to dementia.

Moreover, the performance's effect on Orientation, Delayed Recall, or Luria's clock tests are interestingly similar in those MCI subjects with the presence of at least one ε4 allele and those without ε4 allele. That is, neuropsychological capability of conversion to dementia is independent of being a carrier or not of one ε4 allele.

A limitation of the present study was that cerebrospinal fluid (CSF) biomarkers were not available. However, taking into account that our study aimed to detect prodromal dementia, not only AD, it was not a crucial issue. Moreover, CSF biomarker analysis was not a mandatory technique in Spain for MCI diagnosis when this project was conducted. Even today, lumbar puncture is not mandatory for MCI diagnosis in the clinical routine in our country.

However, we are currently carrying out a further prospective and longitudinal study using this MCI subtypes classification, with CSF biomarkers available.

In summary, follow-up analysis suggested that the probable amnestic-storage group had the highest risk of conversion to dementia, having 8.5 times more risk to convert to dementia than the possible non-amnestic MCI group, the group that resulted in the slowest conversion to dementia. However, our study demonstrates high conversion rates in all MCI categories which strongly support a therapeutic intervention in Probable amnestic-storage individuals (that is, with recognition memory impairment), orientation, impairment, and multiple domain impairment, because almost all of them convert to dementia, especially to AD.

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Supplementary Data

A Longitudinal Follow-Up of 550 Mild Cognitive Impairment Patients: Evidence for Large Conversion to Dementia Rates and Detection of Major Risk Factors Involved

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Supplementary Table 1
Significant predictors of conversion to dementia identified by the forward stepwise (Wald) survival analysis

Forward stepwise (Wald)	B	SE	Wald	df	*p*	OR	[95% CI]
Pr-aMCI							
Neuropsychological variables							
Orientation	−0.17	0.08	4.55	1	0.033	0.84	[0.72, 0.99]
Learning	−0.06	0.03	4.65	1	0.031	0.94	[0.88, 0.99]
Delayed recall	−0.27	0.13	4.01	1	0.045	0.76	[0.59, 0.99]
Recognition memory	−0.08	0.05	2.82	1	0.093	0.92	[0.84, 1.01]
MPI (NS)	−0.41	0.31	1.80	1	0.179	0.66	[0.36, 1.21]
DPI	−0.74	0.39	3.62	1	0.057	1.48	[0.22, 1.02]
APOE genotype (NS)	0.30	0.24	1.67	1	0.197	1.35	[0.86, 2.14]
Pss-aMCI							
Neuropsychological variables							
Orientation	−0.22	0.08	8.18	1	0.004	0.80	[0.69, 0.93]
Delayed recall	−0.19	0.09	4.54	1	0.033	0.83	[0.69, 0.98]
Luria's Clocks test	−0.23	0.09	6.35	1	0.012	0.79	[0.66, 0.95]
SVF	−0.07	0.03	4.40	1	0.036	0.93	[0.88, 0.99]
MPI (NS)	−0.11	0.19	0.34	1	0.562	0.90	[0.62, 1.30]
DPI (NS)	−0.27	0.33	0.64	1	0.422	1.77	[1.40, 1.47]
APOE genotype	−0.64	0.21	9.74	1	0.002	1.90	[1.27, 2.84]
Pr-naMCI							
Neuropsychological variables							
Orientation	0.89	0.47	3.58	1	0.059	2.44	[0.97, 6.15]
Recognition memory	−0.80	0.35	5.18	1	0.023	0.45	[0.22, 0.89]
DPI (NS)	0.18	0.58	0.09	1	0.761	1.19	[1.38, 3.72]
APOE genotype (NS)	−0.82	0.87	0.88	1	0.347	0.44	[0.08, 2.43]
Pss-naMCI							
Neuropsychological variables							
Orientation	−0.48	0.15	10.57	1	0.001	0.62	[0.46, 0.83]
Delayed recall	−0.35	0.16	4.67	1	0.031	0.70	[0.51, 0.97]
Luria's Clocks test	−0.40	0.17	5.69	1	0.017	0.67	[0.48, 0.93]
DPI (NS)	−0.67	0.49	1.90	1	0.168	0.51	[0.20, 1.33]
APOE genotype (NS)	0.52	0.36	2.05	1	0.152	1.57	[0.83, 3.39]

Orientation, summary of Temporal + Spatial + Personal orientation; Learning, Trials 1 + 2 + 3 + 4; Recognition memory, correct answers; SVF, semantic verbal fluency; MPI, memory pattern impairment; DPI, domain pattern impairment; *APOE* genotype, carriers versus non-carriers of at least one $\varepsilon 4$; B, beta coefficient; SE, standard error; Wald, Wald statistical test; df, degrees of freedom; *p*, level of statistical significance; NS, $p \geq 0.10$; OR, odds ratio; 95% CI for OR: 95% confidence intervals for effect size.

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Erratum

A Longitudinal Follow-Up of 550 Mild Cognitive Impairment Patients: Evidence for Large Conversion to Dementia Rates and Detection of Major Risk Factors Involved

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On page 778, in the Acknowledgments, this line is missing:

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The corrected Acknowledgment should read as follows:

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Artículo II

6.2. Cognitive Composites Domain Scores related to Neuroimaging Biomarkers within Probable-amnestic Mild Cognitive Impairment-storage subtype.

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Cognitive Composites Domain Scores Related to Neuroimaging Biomarkers within Probable-Amnestic Mild Cognitive Impairment-Storage Subtype

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Abstract. The probable-amnestic (Pr-a) mild cognitive impairment (MCI)-storage subtype is a phenotype with 8.5 times more risk of conversion to dementia, mainly Alzheimer's disease (AD), than the possible non-amnestic (Pss-na) MCI. The aim of this study was to find the optimized cognitive composites (CCs) domain scores most related to neuroimaging biomarkers within Pr-aMCI-storage subtype patients. The Fundació ACE (ACE) study with 20 Pr-aMCI-storage subtype subjects (MCI) were analyzed. All subjects underwent a neuropsychological assessment, a structural MRI, FDG-PET, and PIB-PET.

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The adjusted hippocampal volume (aHV) on MRI, the standard uptake value ratio (SUVR) on FDG-PET and PIB-PET SUVR measures were analyzed. The construction of the CCs domain scores, and the aHV on MRI and FDG-PET SUVR measures, were replicated in the parental AB255 study database ($n = 133$ MCI). Partial correlations adjusted by age, gender, and education were calculated with the associated p-value among every CC domain score and the neuroimaging biomarkers. The results were replicated in the "MCI due to AD" with memory storage impairments from ADNI. Delayed Recall CC domain score was significantly correlated with PIB-PET SUVR ($\beta = -0.61$, $p = 0.003$) in the ACE study and also with aHV on MRI ($\beta = 0.27$, $p = 0.01$) and FDG-PET SUVR ($\beta = 0.27$, $p = 0.01$) in the AB255 study. After a median survival time of 20.6 months, 85% from the ACE MCI converted to AD. The replication of our results in the ADNI dataset also confirmed our findings. Delayed Recall is the CC domain score best correlated with neuroimaging biomarkers associated with prodromal AD diagnosis.

Keywords: Alzheimer's disease, amnestic mild cognitive impairment, amyloid, cognition, hippocampus, magnetic resonance imaging, memory, positron emission tomography

INTRODUCTION

The search for reliable Alzheimer's disease (AD) biomarkers to identify prodromal AD [1, 2], or mild cognitive impairment (MCI) due to AD [3], already implemented or that has been proposed should be used in clinical trials and clinical diagnosis [1, 3–7], has focused on expensive methods which are often poorly tolerated by patients (e.g., PET). By contrast, neuropsychological tests are non-invasive, and may be cost-effective, better predictors of disease than neuroimaging [8]. The identification of optimal cognitive composites (CCs) domains that are related to neuroimaging biomarkers and that characterize specific phenotypes will maximize the cost-effectiveness of clinical practice and management, and recruitment into clinical trials.

In a recent study [9], at *The Memory Disorders Unit* from Fundació ACE (Barcelona, Spain) [10] which involved the follow-up of 550 MCI subjects for an average of 26.6 months, the present authors found that the majority (45.5%) of those MCI individuals who subsequently developed dementia displayed the AD dementia phenotype.

The MCI subjects were classified as probable/possible in function of the presence of comorbidities that could otherwise explain their cognitive deficits [9, 11–13]. Among those probable-amnestic (Pr-a) MCI patients with memory storage impairment [14] (i.e., impaired recall and recognition), there was an 8.5 times greater risk to develop dementia, mainly AD, than those with the possible MCI condition, where cognitive deficits did not include memory.

The aim of the present study was to find the optimized CC domain scores that were most related to three brain imaging biomarkers, derived from structural magnetic resonance imaging (MRI), [^{18}F]-fluorodeoxyglucose-positron emission tomography (FDG-PET), and Pittsburgh compound B-positron emission tomography (PiB-PET) within the Pr-aMCI storage phenotype.

MATERIALS AND METHODS

Subjects

For the purpose of this study, two groups of datasets, >64 years old, were analyzed: (i) The Fundació ACE (ACE) study ($n = 59$) with 20 Pr-a-MCI-storage subtype subjects (MCI, from now on) (60.0% men) all with multiple domains impaired and 39 healthy controls (HC) (51.3% men), were all recruited and assessed from 2010 to 2013 at *The Memory Disorders Unit* from Fundació ACE, Institut Català de Neurociènces Aplicades, Barcelona, Spain [10]. All subjects underwent a neuropsychological assessment including five cognitive domains and were subjected to a structural MRI, FDG-PET, and PIB-PET; and (ii) The parental AB255 study ($n = 175$) with 133 MCI all with multiple domains impaired and 42 HC, were all recruited and assessed at 19 clinical memory research sites in Spain, Italy, Sweden, and France and led by Araclon Biotech S.L., Zaragoza, Spain. The construction of the five CCs domain scores, and the adjusted hippocampal volume (aHV) on structural MRI, and the standard uptake value ratio (SUVR) on FDG-PET measures, were replicated in the parental AB255 study database.

The study was approved by the CEIC ethic committee 2009/5455, and all participants provided written informed consent prior to inclusion.

Clinical characteristics of the ACE study and the AB255 study

All had the following inclusion criteria: aged from 65 to 85; without lifetime history of psychiatric, neurological, or systemic illnesses; autonomy for instrumental activities of daily living; preserved global cognition (Mini-Mental State Examination, MMSE) [15, 16] (≥ 24 scores); with general good health; a Hachinski Ischemia Scale [17] score ≤ 4; without depressive symptoms measured by self-rating Geriatric Depression Scale [18] (< 11 scores); at least minimal elementary school; without severe auditory or visual abnormalities including glaucoma and cataracts; and, DNA sample available. Those subjects with significant vascular pathology on MRI that could explain memory deficits, and/or with contraindications for neuroimaging administration, were excluded from the study.

Diagnostic adjudication

The HC subjects had a normal clinical history for their age, and no neurological signs or symptoms. They did not report problems with memory or other cognitive functions, and their performance on the MMSE [15, 16], and measures of memory function were normal. The Clinical Dementia Rating (CDR) [19] was 0, and none had a family history of dementia.

The MCI patients fulfilled Petersen's diagnostic criteria [20], including subjective memory complaint, relatively preserved performance in activities of daily living, absence of dementia, and a measurable impairment in memory function, with or without a deficit in other cognitive domains [21]. They did not have significant clinical comorbidities (i.e., cerebrovascular disease, history of head trauma encephalopathy, infectious diseases, or developmental disabilities) that could themselves cause cognitive deficits [12, 13]. The memory loss was characterized as being one of impaired storage [14] because both verbal delayed recall and recognition memory were impaired. The CDR score was 0.5, with a 0.5 or 1 score for memory; the Interview for Deterioration in Daily Activities in Dementia score was less than 40 [22]. Those subjects who converted to dementia, that is, to AD [5, 23], and mixed dementia (AD with cerebrovascular disease) over the study period, were classified as MCI converters. All of them had a CDR [19] of 1. In contrast, those subjects who remained stable during follow-ups were classified as stable or non-MCI converters.

Neuropsychological assessment

All subjects underwent a neuropsychological battery for diagnostic purposes, including evaluation of i) global cognition using the MMSE [15, 16], once adjusted by age and educational level; ii) verbal learning and memory by The Word List Learning test from the Wechsler Memory Scale-Third Edition (WMS-III) [24], including delayed recall, and a recognition task) without list of interference [25, 26], and the Free and Cued Selective Reminding Test (FCSRT) [27] adjusted by age and educational level; and, iii) The Vocabulary test of the Wechsler Adult Intelligence Scale-Third Edition (WAIS-III) [28], as a previous cultural level estimation tool.

Comparison of demographic, genetic, and neuropsychological data of these subjects are detailed in Table 1. There were no statistically significant differences between HC and MCI subjects in education or gender, but they did differ in age (Cohen's $d = 1.00$, $p = 0.001$) (Table 1). The MCI patients had a higher frequency of APOE $\varepsilon 4$ allele carriers (presence of at least one $\varepsilon 4$ allele) compared to the HC subjects ($\chi^2 = 22.26, p < 0.001$, Odds Ratio = 20.42). The MCI patients had significantly lower scores in the Global Cognition, Verbal Memory, and Vocabulary test compared to the HC group (Table 1).

Cognitive composites construction

All subjects underwent a neuropsychological battery with tests in five cognitive domains: 1) *Memory*: Rey's Auditory-Verbal Learning Test (RAVLT) [29] and Alzheimer's Disease Assessment Scale-Cognitive Subscale (ADAS-Cog) [30] (only immediate recall on memory condition); 2) *Delayed Recall:* both verbal RAVLT [29] and ADAS-Cog [30] also incorporating non-verbal (Rey-Osterrieth Complex Figure Test, ROCF) [29]; 3) *Processing Speed, Attention, and Executive Functions:* Digit Symbol coding and Digit spans forwards and backwards [29], Trail Making Test (part B-part A) [29]; Semantic Verbal Fluency ("animals" during one minute) [29], and Phonetic Verbal Fluency (words beginning with "P" during one minute) [29]; 4) *Language:* Boston Naming Test [29] and Commands item from the ADAS-Cog [30]; and 5) *Praxis:* the ROCF copy condition [29] and Block Design from WAIS-III [29].

In order to create the CCs domain scores, the data were first analyzed by a principal component analysis (PCA). Five separate PCAs were performed, one for each of the following cognitive domains: 1)

Table 1
Demographic, genetic and neuropsychological data between HC and MCI subjects from the ACE study and the AB255 study

	HC	MCI	Statistics	*p*	Effect size[3]
N (%)	39 (66.1)	20 (33.9)			
	42 (24.0)	*133 (76.0)*			
Gender *n* (%) Male	20 (51.3)	12 (60.0)	0.59[1]	0.360	0.70
	21 (50.0)	*64 (48.1)*	*0.04*[1]	*0.832*	*1.08*
Education in years	12.3 ± 4.1	10.6 ± 4.1	2.01[2]	0.161	0.42
	12.2 ± 3.9	*15.4 ± 22.9*	*0.81*[2]	*0.370*	*0.24*
Age in years	71.3 ± 4.4	75.7 ± 4.4	13.15[2]	0.001**	1.00
	71.0 ± 4.5	*74.0 ± 5.1*	*11.89*[2]	*0.001***	*0.63*
APOE 4 *n* (%)	4 (10.3)	14 (70.0)	22.26[1]	0.001**	20.42
(presence of ε4 or ε4/ε4)	*4 (9.5)*	*73 (54.9)*	*26.66*[1]	*0.001***	*11.56*
Neuropsychological battery					
Global Cognition					
MMSE	29.7 ± 0.6	26.0 ± 1.9	43.09[2]	0.001**	2.96
	29.7 ± 0.6	*26.2 ± 2.2*	*104.09*[2]	*0.001***	*2.50*
Verbal Memory					
Verbal Learning and Memory WMS-III					
Learning (Trials 1+2+3+4)	30.0 ± 5.2	16.0 ± 4.9	32.79[2]	0.001**	2.77
	29.6 ± 5.1	*16.6 ± 4.8*	*227.90*[2]	*0.001***	*2.63*
Delayed Recall	6.3 ± 2.8	0.3 ± 0.7	33.85 [2]	0.001**	3.43
	6.1 ± 2.5	*1.2 ± 1.7*	*210.04* [2]	*0.001***	*2.33*
Recognition memory	22.3 ± 1.8	14.6 ± 2.7	53.84[2]	0.001**	3.42
	22.1 ± 1.8	*15.3 ± 2.1*	*350.73*[2]	*0.001***	*3.49*
Buschke Selective Reminding Test					
Free recall (Trials 1+2+3)	23.7 ± 6.7	3.1 ± 3.0	58.80[2]	0.001**	4.25
	23.7 ± 6.7	*6.1 ± 4.7*	*359.54*[2]	*0.001***	*3.09*
Cued Recall (Trials 1+2+3)	18.9 ± 5.2	10.6 ± 6.2	12.11[2]	0.001**	1.46
	18.5 ± 5.0	*11.9 ± 6.1*	*40.90*[2]	*0.001***	*1.19*
Free + Cued Recall	42.5 ± 4.4	13.8 ± 7.9	107.15[2]	0.001**	4.67
	42.7 ± 4.4	*17.9 ± 8.9*	*289.76*[2]	*0.001***	*3.73*
Vocabulary test (WAIS-III)	13.9 ± 2.1	12.4 ± 2.4	4.11[2]	0.011*	0.88
(Scalar Scores)	*13.8 ± 2.1*	*11.8 ± 2.5*	*32.58*[2]	*0.001***	*1.05*

Values in regular print correspond to ACE study and values in *italic* correspond to AB255 study; HC, healthy controls; MCI, amnestic mild cognitive impairment-storage type; MMSE, Mini-Mental State Examination; WMS-III, Wechsler Memory Scale, Third Edition; Recognition memory, correct answers WMS-III; WAIS-III, Wechsler Adult Intelligence Scale-Third Edition. Values reported are means:±; SD, standard deviation; [1]: χ^2; [2]: F; [3]: Cohen's *d* for continuous variables, and Odds ratio for categorical data are reported. *Statistically significant $p \leq 0.01$; **$p \leq 0.001$.

learning, 2) delayed recall on memory, 3) executive functions, 4) language, and 5) praxis. Every PCA was forced to produce a unidimensional factorial solution, according to the expected unidimensional neuropsychological function assessed. The original variables contributed to the final score in a weighted way, based on the magnitude of the inter-correlations among the variables in the same composite domain. The stability of the PCA was evaluated by means of the Hotelling's T2 test. When a neuropsychological variable obtained a factorial loading <0.3 in the one-dimension solution, the variable was excluded from the analysis [31], assuming that this variable has a poor empirical contribution to the corresponding inferred cognitive function. According this criterion, Digit Symbol coding, Digit spans forwards, and backwards subtests of WAIS-III [29], and Commands from ADAS-Cog [30], were each excluded from the corresponding PCA analyses. The linear function of the original variables from the factorial solution, was used as a final standardized domain score, for each subject, which we identified as a composite in this study. Each cognitive domain corresponded to a CC domain score that could be later analyzed using standard procedures.

Neuroimaging acquisition and analysis

All subjects from the ACE study underwent a structural MRI, FDG-PET, and PiB-PET within 30 days following the neurological and neuropsychological visits. All subjects from the AB255 study underwent a structural MRI and FDG-PET. Imaging data were analyzed using the Fundació ACE Pipeline for Neuroimaging Analysis, available at http://detritus.fundacioace.com/.

MRI

All MRI scans were performed with a 1.5T MR scanner (Magnetom Symphony; Siemens Medical Solutions, Erlangen, Germany). The protocol for the acquisition of the MRI data was identical for all subjects and consisted of 3D T1-weighted Sagital MP-RAGE, 2D Axial T2-weighted TSE, 2D Axial Fluid-Attenuated Inversion Recovery (FLAIR), 2D Axial T2* Gradient Echo and 2D Axial Diffusion Weighted Imaging. Brain images were also visually inspected by experienced clinicians who were blinded to the participants' demographic, anthropometric, and clinical data. All MRI were acquired before the PET and an expert neuroradiologist excluded any form of vascular pathology over participants. Subjects with MRI evidence of major stroke, white matter hyperintensities, leukoaraiosis, and lacunae were excluded.

Cortical reconstruction and volumetric segmentation was performed with the Freesurfer 5.3 image analysis suite, which is documented and freely available for download online (http://surfer.nmr.mgh.Harvard.edu/). The technical details of these procedures are also described in prior publications [32, 33]. Freesurfer morphometric procedures have been proven to show good test-retest reliability across scanner manufacturers and across field strengths [34, 35]. The procedures for the measurement of cortical thickness have been validated against histological analysis [34] and manual measurements [33, 36].

A residual approach was used to adjust hippocampal volume by total intracranial volume (ICV) [37]. The aHV was obtained with the following formula: aHV = HV-b (ICV-<ICV>), where HV is the mean value between right and left HV, <ICV> reflects the group mean ICV value and b is the regression slope between ICV and HV.

FDG-PET

The FDG-PET were acquired 60 min after intravenous injection of approximately 370 MBq of [^{18}F]-FDG during 20 min. The imaging processing and calculation of mean value for the defined ROI were performed using the imaging processing suite FSL, free available online at http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/. Every individual scan was averaged and corrected. Then, the resultant images were coregistered to Montreal National Institute (MNI) standard space and mean value of FDG-PET activity calculated for a composite region-of-interest (ROI). This composite ROI was built with a set of five ROIs (left and right angular gyri, bilateral posterior cingulate gyrus, and left middle and inferior temporal gyrus) based on coordinates cited frequently in other FDG studies comparing AD, MCI, and normal subjects in the ADNI (Alzheimer's Disease Neuroimaging Initiative) database and available in http://adni.loni.usc.edu/methods/research-tools/. The SUVR was calculated normalizing by the value of vermis/pons as described in Landau et al. [38].

Notice that volumetric and FDG-PET analysis carried on our data were made using the same methods that ADNI site recommend. Information about these analyses are available in ADNI website (http://adni.loni.usc.edu/methods/).

PIB-PET from the ACE study

All these individuals also received a PIB-PET scan. The PIB-PET was acquired 50 min after intravenous injection of 400 MBq of the radiotracer. Every subject MRI was previously segmented using Freesurfer. A cortical composite ROI was built with the labels exported by Freesurfer segmentation on Desikan-Killiany Atlas. The composite ROI included four large cortical grey matter regions (frontal, anterior/posterior cingulate, lateral parietal, lateral temporal). The SUVR was calculated using the cerebellum as reference region [39, 40].

Apolipoprotein E (APOE) genotyping

APOE genotyping was performed for all subjects as previously described by Hixson et al. [41] using the amplification of genomic DNA, digestion with HhaI, and further analysis of the restriction fragments.

Alzheimer's disease neuroimaging initiative data

Additional data used in the preparation of this study were obtained from the ADNI database (http://adni.loni.usc.edu). The ADNI was launched in 2003 as a public-private partnership, with the primary goal to assess relationships between serial MRI, PET, other biological markers, and clinical and neuropsychological assessment in the progression of MCI and early AD.

The ADNI repository was explored for MCI patients with PIB-PET images ($n = 65$) [42, 43], and who were similar in age, gender, and educational levels to the ACE study and in parental dataset the

Table 2
Partial correlations between neuroimaging biomarkers and the five CCs domain scores in MCI subjects from the ACE study ($n = 20$) and the AB255 study ($n = 133$)

CCs related to:	(A) aHV		(B) FDG-PET		(C) PIB-PET	
	β	*p*-value	β	*p*-value	β	*p*-value
Learning CC	0.09	0.74	0.06	0.82	– 0.02	0.92
	0.13	*0.12*	*0.05*	*0.59*	*NA*	*NA*
Delayed Recall CC	– 0.02	0.94	0.06	0.82	– 0.61	0.003*
	0.27	*0.01**	*0.27*	*0.01**	*NA*	*NA*
Executive Functions CC	0.29	0.24	0.23	0.37	– 0.19	0.45
	0.01	*0.92*	*0.21*	*0.03*	*NA*	*NA*
Language CC	0.23	0.37	– 0.04	0.87	0.21	0.40
	0.06	*0.51*	*0.04*	*0.65*	*NA*	*NA*
Praxis CC	0.19	0.46	– 0.01	0.97	– 0.07	0.79
	–0.08	*0.33*	*0.13*	*0.14*	*NA*	*NA*

Values in regular print correspond to ACE study and values in *italic* correspond to AB255 study; CC, Cognitive Composite domain score; aHV, adjusted hippocampal volume; β, partial correlations adjusted by age, gender, and education; NA, not applicable; *Statistically significant after Bonferroni's correction $p \leq 0.01$.

AB255 study. From those with MCI, 54 subjects (34 PIB positive and 16 PIB negative) were labeled as "MCI due to AD" with memory storage impairment. Moreover, those MCI with structural MRI ($n = 477$) and with FDG-PET ($n = 336$) were also downloaded from the ADNI repository for a later comparison of results obtained in this study.

The subtests used for the construction of the ADNI CC domain score were the delayed recall on memory scores from ADAS-Cog scale and RAVLT. ADNI PIB neuroimaging analysis was completed at University of Pittsburgh, PET Facility. The methods used in this analysis are described at the ADNI website (http://adni.loni.usc.edu/methods/pet-analysis).

An automated template-based method was used to sample multiple ROIs on the ADNI PIB SUVR image. The PIB SUVR was downloaded from the ADNI website along with its corresponding ADNI Processed 3 MR image. The MR image choice was scanner dependent. The PIB SUVR image has been co-registered to the first frame of the raw image file and averaged across frames (for dynamic acquisitions only), reoriented to Talairach space, intensity normalized so that the average of voxels within the mask was exactly 1, and smoothed to achieve a uniform isotropic resolution of 8 mm FWHM. A set of four ROIs were averaged to calculate the PiB SUVR in the frontal cortex, anterior cingulate, parietal cortex and precuneus. All the values were normalized to the cerebellum uptake value. A description of the other variables used is detailed in Supplementary Table 1. Notice that the procedures to calculate the PIB SUVR by the University of Pittsburgh at ADNI database and along this study are completely different. So the data could not be merged but any comparison must be done between the final results.

Statistical analysis

Partial correlations, adjusted by age, gender, and educational level, were carried out among every CC domain score and the three neuroimaging biomarkers, the aHV on MRI, FDG-PET SUVR, and PIB-PET SUVR. This procedure was performed for both the whole sample (the MCI and the HC groups) and the MCI group alone. The PIB-PET SUVR was compared to the aHV and FDG-PET SUVR in order to contrast amyloid-β (Aβ) burden within the MCI group, related to the significant CCs domain scores. Further, a Kaplan-Meier survival analysis was executed in order to estimate survival times in MCI from the ACE study. The aHV on MRI and FDG-PET results from the AB255 study and PIB-PET results from ACE study were replicated using data from the ADNI website (http://adni.loni.usc.edu) and specifically, R ADNIMERGE package (http://adni.bitbucket.org). Cox proportional hazards with adjustments for age, gender, and education were also completed from ADNI data.

RESULTS

As can be seen in Supplementary Table 2, the correlations between the three neuroimaging biomarkers and the five CCs domain scores are strong for the whole sample from the ACE study and the AB255 study.

This was expected since MCI and HC groups are quite different for biomarkers and neuropsychological CCs domains scores.

Within the ACE MCI study, there was only a single significant partial correlation between PIB-PET

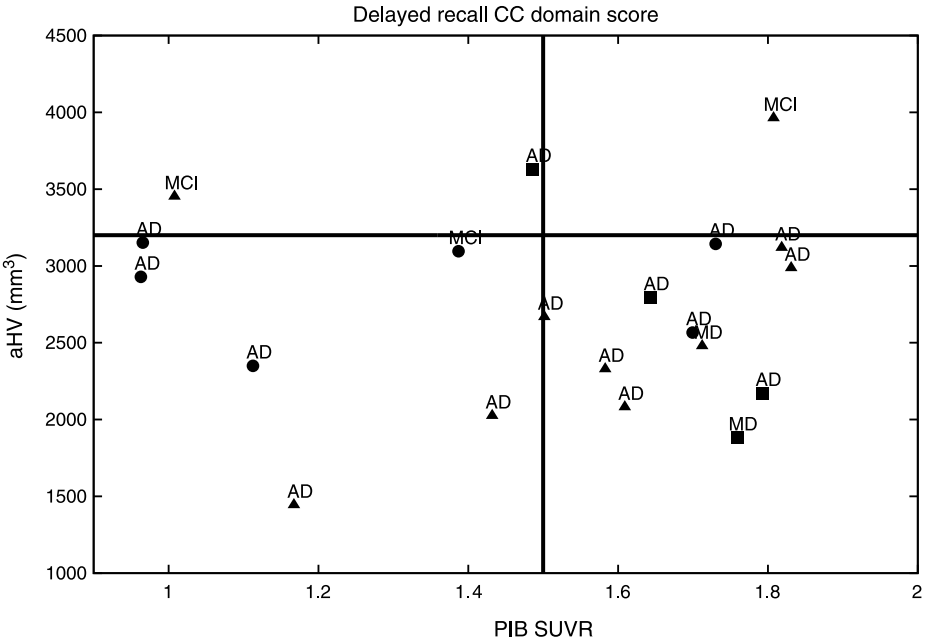

Fig. 1. PIB SUVR related to the Delayed Recall CC established by aHV in the MCI group. [a]The MCI subjects with the best performances on the Delayed Recall CC domain score are shown with circles, [b]those with intermediate performances are represented with triangles, [c]while those with the worst performances are shown with squares. [d]After a median survival time of 20.6 months (SD: 15.5; range: 6–68), 17/20 (85%) of the MCI patients developed dementia; 15 (88.2%) with AD; and 2 (11.8%) with a mixed dementia (AD with cerebrovascular disease).

SUVR and the Delayed Recall CC domain score ($\beta = -0.61$, $p = 0.003$) (Table 2C).

In our replication MCI sample from parental dataset AB255, there was a significant partial correlation between the aHV and the Delayed Recall CC domain score ($\beta = 0.27$, $p = 0.01$) (Table 2A), and between the FDG-PET SUVR and Delayed Recall CC domain score ($\beta = 0.27$, $p = 0.01$) (Table 2B).

MCI subjects from the ACE study demonstrated an Aβ burden related to the Delayed Recall CC domain score as established by aHV on MRI (Fig. 1) and FDG-PET (Fig. 2). They were classified into aHV+ ($n = 17$) and aHV- ($n = 3$) with a threshold of aHV = 3.2 cm^3, and into FDG+ ($n = 18$) or FDG- ($n = 2$) with a threshold of FDG-PET SUVR = 1.3. Those thresholds were the biomarker values that better separated the HC and MCI groups for each case. The Aβ burden cut-off was taken as PIB-PET SUVR = 1.5, for 12 PIB+ and 8 PIB- subjects. The Delayed Recall CC domain score, naturally divided the sample into positive and negative subjects by its mean value of zero. Indeed, this is the threshold that best separated HC and MCI groups.

After a median survival time of 20.6 months (SD: 15.5; range: 6–68), 17/20 (85%) of the ACE MCI patients developed dementia; 15 (88.2%) with AD and 2 (11.8%) with a Mixed Dementia (AD with cerebrovascular disease) (Figs. 1 and 2). Over the course of the observation, one of the MCI patients died and was censored for the analysis of dementia incidence. Among this group, Kaplan-Meier analyses showed that the Delayed Recall CC was the best domain score to predict conversion to dementia compared to the other CCs (see Supplementary Table 3 and Supplementary Figure 1 for details); however, this only showed a tendency to approach significance (Wald = 3.49; $p = 0.06$; Odds Ratio = 7.40) (0.91–58.82 [95% CI]).

Finally, the correlation between the five CCs domain scores and the three biomarkers was replicated using the ADNI dataset (Table 3). The partial correlations between the Delayed Recall CC domain score and aHV on MRI (Table 3A), FDG-PET (Table 3B), and PIB (Table 3C) were the most significant in MCI from the ADNI dataset.

After a median survival time of 6.5 months (SD: 5.4; range: 6 months-4years), 26/54 (48%) of the ADNI MCI patients developed dementia, specifically AD.

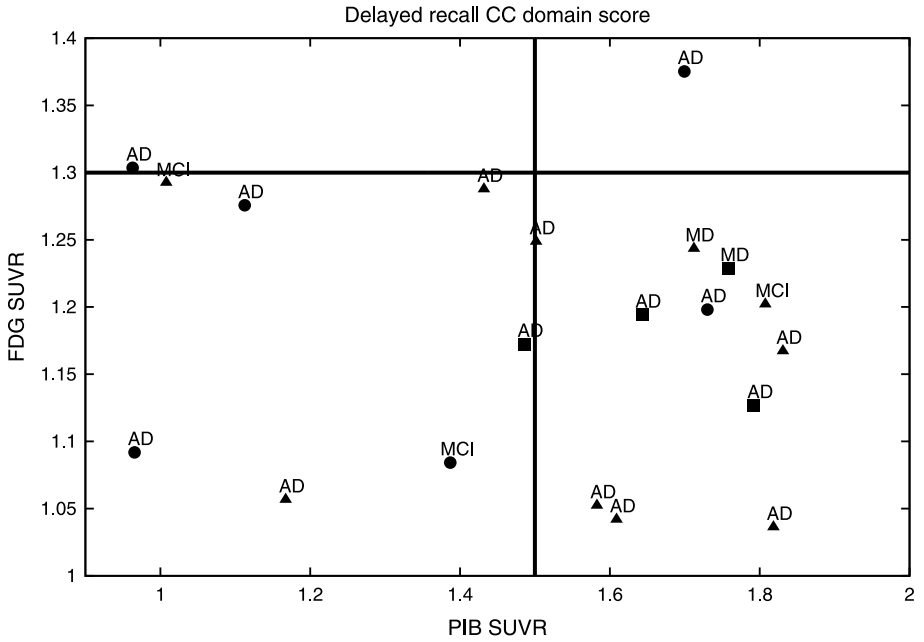

Fig. 2. PIB SUVR related to the Delayed Recall CC established by FDG in the MCI group. [a]The MCI subjects with the best performances on the Delayed Recall CC domain score are shown with circles, [b]those with intermediate performances are represented with triangles, [c]while those with the worst performances are shown with squares. [d]After a median survival time of 20.6 months (SD: 15.5; range: 6–68), 17/20 (85%) of the MCI patients developed dementia; 15 (88.2%) with AD; and 2 (11.8%) with a mixed dementia (AD with cerebrovascular disease).

Table 3
Partial correlations between neuroimaging biomarkers and the five CCs domain scores in MCI subjects from the ADNI dataset

CCs related to:	(A) aHV		(B) FDG-PET		(C) PIB-PET	
	β	*p*-value	β	*p*-value	β	*p*-value
Learning CC	0.35 ($n=477$)	2.95 e–15**	0.23 ($n=336$)	1.51 e–05**	– 0.39 ($n=54$)	0.004
Delayed Recall CC	0.50 ($n=477$)	1.56 e–31**	0.30 ($n=336$)	2.17 e–08**	– 0.42 ($n=54$)	0.002*
Executive Functions CC	0.18 ($n=479$)	5.98 e–05**	0.29 ($n=334$)	4.88 e–08**	– 0.35 ($n=54$)	0.01
Language CC	0.14 ($n=478$)	0.002*	0.14 ($n=337$)	0.01	– 0.19 ($n=54$)	0.19
Praxis CC	NA	NA	NA	NA	NA	NA

CC, Cognitive Composite domain score; aHV, adjusted hippocampal volume; β, partial correlations adjusted by age, gender, and education; *Statistically significant after Bonferroni's correction $p \leq 0.01$; **$p \leq 0.001$; NA, not applicable on ADNI.

Within MCI with PIB-PET, Cox proportional hazards with adjustments for age, gender, and education showed that the Delayed Recall CC significantly predicted conversion to AD dementia (Wald = 11.32; $p = 0.001$; Odds Ratio = 2.51) (1.46 – 4.29 [95% CI]) (see Supplementary Figure 2 for details).

DISCUSSION

This work explored the relationship between five CCs domain scores, and three AD neuroimaging biomarkers covering neurodegeneration and Aβ deposition, within probable MCI [12, 13] subjects with memory storage impairment [9]. As previously reported [9], the probable MCI condition had higher risk of early conversion to dementia, mainly AD than the rest of MCI individuals. Remarkably, those Pr-aMCI subjects with storage memory impairment had the most and closest risk of conversion to dementia, mainly AD compared to the other MCI subtypes.

Of note, since the aim of our study was include only the Pr-aMCI-storage subtype subjects, we reported as a unique condition that they must all have a memory loss characterized as being one of impaired storage, that is, independently if they had single or multiple

domains impaired. However, all MCI had deficits in multiple cognitive domains. Our data supports that the traditional amnestic single-domain aMCI (aMCI-sd) [20] is rarely diagnosed when a comprehensive neuropsychological battery is applied, because other cognitive deficits are frequently found when neuropsychological evaluation is expanded [44].

One of our central goals was to extend the limited literature of CCs domain scores combined with neuroimaging biomarkers in this population. In particular, focus on the relation with Aβ burden, discussed in more detail later. Then we analyzed the correlation between these two magnitudes. We found that only poorer performances on Delayed Recall CC domain score are related to reduced hippocampal volume, greater hypometabolism, and also greater Aβ burden. Other CCs domain scores, such as executive functions, language, and praxis, were not found to be related to neuroimaging biomarkers used. Our results are according to previous studies that reported that poorer Delayed Recall was related to reduced hippocampal volume as well as greater hypometabolism in MCI patients who were near to AD dementia conversion [45–51].

However, our main finding is that the Aβ burden of the MCI subjects, related to the Delayed Recall CC domain score performances, did not allow classifying all MCI subjects as Prodromal AD in either aHV on MRI or FDG-PET. Instead, some of them had high and other low Aβ burden. Importantly, that as mentioned above, we included subjects in the worst case situation in order to distinguish it, understanding that the MCI sample, all Pr-aMCI-storage subtype, has a high homogeneity phenotype, and besides this, contains some members with closely AD-like cognitive and biomarker pattern who are likely to convert in the near future. Furthermore, observed APOE $\varepsilon 4$ allele enrichment in the ACE study (70.0%), closely resembled histopathological series reported in AD [52]. In fact, almost all of MCI converted to dementia, mainly developed clinical AD.

As a fact, while all subjects were positive at baseline for at least one neuroimaging biomarker, only 11 (55%) subjects were positive for the three biomarkers at the same time. In contrast, 17 (85%) subjects were positive for aHV, 18 (90%) were positive for FDG-PET, and 12 (60%) were positive for PIB-PET. Also, 15 (75%) subjects were positive for aHV and FDG-PET, 11 (55%) were positive for aHV and PIB-PET, and 11 (55%) were positive for FDG-PET and PIB-PET. Regarding the Delayed Recall CC domain score, all subjects had a negative value.

Hence, Pr-aMCI-storage subjects with higher Aβ burden could be considered canonical prodromal AD (represented in the right bottom corner of both figures). All of the subjects with both greater hippocampal atrophy and hypometabolism could find representation in the biomarker model of pure AD [53–56]. In contrast, it is likely that MCI subjects with lower Aβ burden behave like SNAP (suspected non-Alzheimer's pathology) subjects, i.e., those individuals with neuroimaging/biomarker evidence of AD-like neurodegeneration without clinical amyloidosis [57] (represented in the left bottom corner of both figures). All of these subjects could find representation on the neurodegeneration-first biomarker model of late-onset AD [53–56]. Note that one subject might be showing low performances on the Delayed Recall CC domain score, even before showing high Aβ burden. In addition, other subjects with high Aβ burden do not show enough hippocampal atrophy or hypometabolism to be classified as prodromal AD (top right corner of both figures). All of them could find representation in the amyloid-first biomarker model of late-onset AD [53–56].

Nevertheless, two of our MCI subjects could not find representation in any of the three Jack's models [53–56] (left top corner of both figures). Note that although both developed AD dementia, were in the boundaries to be considered with high Aβ burden based on aHV on MRI, and on FDG-PET SUVR.

For a big fraction of our MCI subjects, clinical and cognitive features that are: the "probable" clinical condition, the memory storage impairment, and the impaired Delayed Recall CC domain score precedes in this study amyloid and neurodegeneration biomarkers. This finding was unexpected given the fact that previous studies with MCI reported that the core biomarker pattern provided clinical evidence of the AD model in patients with MCI [58, 59]. The selection of this MCI population in clinical trials with the poorer performances on the Delayed Recall CC domain score could avoid substantial heterogeneity in biomarkers previously reported within amnestic MCI who were clinically similar [60].

Our main finding of this specific clinical-cognitive phenotype MCI observed before amyloidosis is the first reported in the literature. That is, a big fraction of our MCI subjects that converted to clinical AD dementia have not found representation in line with the current pathophysiologic model of AD; even two of our MCI subjects have not found it in any of the three different sets of biomarker models Jack and colleagues proposed [53–56].

After a median survival time of 20.6 months (SD: 15.5; range: 6–68), 17/20 (85%) of the ACE MCI patients developed dementia; 15 (88.2%) AD type; and 2 (11.8%) mixed dementia (AD with cerebrovascular disease). This points out the importance of follow-up in this MCI group. However, further studies including a more extensive longitudinal clinical follow-up and anatomopathological data would be needed to corroborate these results.

With regard to ADNI, the procedures for the calculation of PIB SUVR and the Delayed Recall CC domain score slightly differed between samples. Also, the composite scores calculated on both datasets are not exactly the same. The corresponding CC domain score for the Delayed Recall differed on the visual memory by the ROCF test from the ADNI and the ACE and the AB255 studies, but both reflects the performance on the same cognitive domain. So although data cannot be merged across datasets, any comparison should be done between the statistical results. In addition, with our ADNI replication, we confirm that the Delayed Recall is the optimal CC domain score to look for prodromal AD, when compared to the other CC domains. Other CCs such as Learning and Executive functions domain scores were found related to hippocampal volume and FDG-PET, previously associated in MCI and AD patients with increasing disease severity [61].

One of the limitations of this study is firstly the lack of amyloid PET in parental dataset AB255; thus, the sample size of subjects with amyloid PET in ACE study was small. This fact could explain that the Delayed Recall CC domain score only showed a tendency to approached significance toward superiority with respect to the other CCs domain scores, as a predictor of conversion to dementia. However, in our replication from ADNI, the Delayed Recall was the best CC domain score as a predictor of conversion to AD. Secondly, we were not able to measure sensitivity and specificity values because of the small sample sizes. Our results are limited to those Pr-aMCI-storage subtype subjects and therefore, are not applicable or extendable to the majority of MCI cases. Further studies incorporating a more heterogeneous MCI group phenotype, i.e., including the possible/probable and amnestic/non-amnestic MCI subtypes [9] could be considered.

In conclusion, according to our results, we strongly suggest selecting those subjects within Pr-aMCI-storage subtype with the worst performances on Delayed Recall CC domain score in order to maximize cost-benefits of clinical trial recruitment.

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SUPPLEMENTARY MATERIAL

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Supplementary Material

Supplementary Table 1. Variables used in the ADNI PIB replication study (mean/standard deviation).

N=54 MCI due to AD (36 male and 18 female)	**Mean**	**SD**
Delayed recall CC	0.00	0.93
PIB-PET	1.83	0.44
Education in years	16.32	2.56
Age	76.27	7.99

Supplementary Table 2. Partial correlations in the whole sample (HC and MCI) from the ACE study and the AB255 study

CCs	aHV related to CC (n=175)		FDG-PET related to CC (n=175)		PIB-PET related to CC (n=59)	
	β	p-value	β	p-value	β	p-value
Learning CC	0.36	1.55 e-08**	0.38	1.00 e-07**	- 0.51	1.26 e-05**
Delayed Recall CC	0.46	9.66 e-14**	0.55	8.56 e-17**	- 0.54	2.24 e-06**
Executive Functions CC	0.32	6.80 e-06**	0.44	4.30 e-09**	- 0.45	0.01*
Language CC	0.31	1.93 e-06**	0.32	8.20 e-06**	- 0.28	0.03
Praxis CC	0.18	0.01*	0.36	5.67 e-07**	- 0.33	0.01*

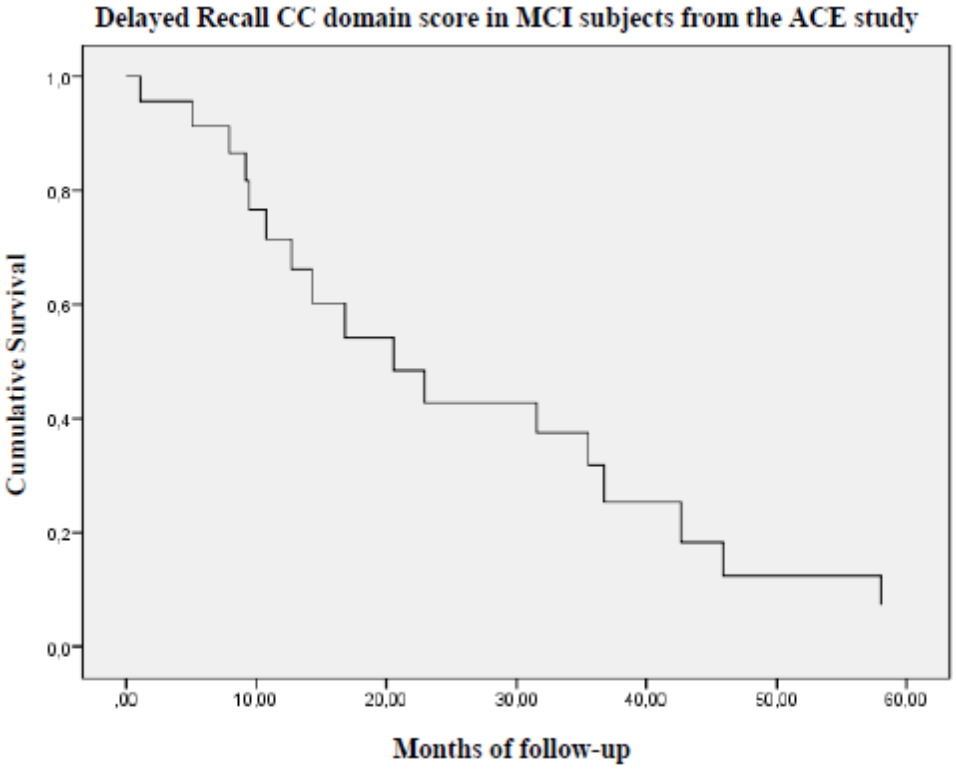
CC, cognitive composite domain score; aHV, adjusted hippocampal volume; β, partial correlations adjusted by age, gender, and education; *Statistically significant after Bonferroni's correction $p \leq 0.01$; **$p \leq 0.001$.

Supplementary Table 3. Survival time of conversion to dementia. Non-converters MCI (n=3) were censored (15%).

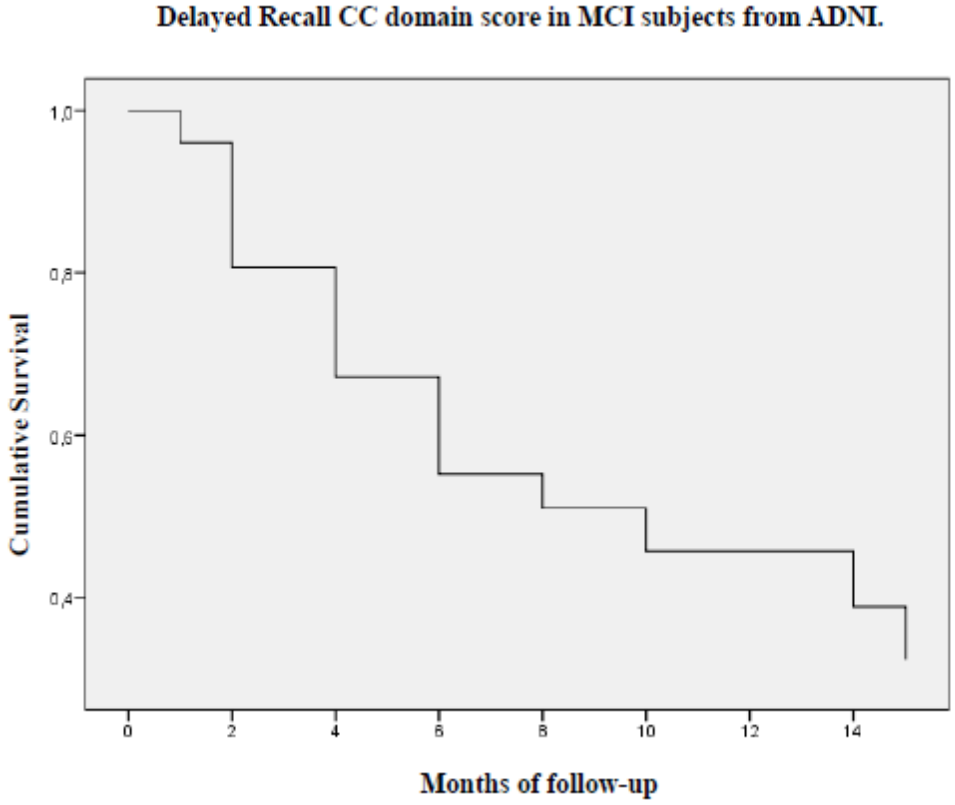
CCs	PIB-PET related to CCs domain scores				
	Wald	**p-value**	**Odds Ratio**	**Inferior CI**	**Superior CI**
Learning CC	0.03	0.86	1.12	0.29	4.29
Delayed Recall CC	3.49	*0.06	7.40	0.91	58.82
Executive Functions CC	3.03	0.08	2.31	0.90	5.95
Language CC	1.36	0.24	1.53	0.75	3.11
Praxis CC	1.12	0.29	1.38	0.76	2.53

CC, cognitive composite domain score; OR, Odds ratio; CI, Confidence Interval at 95%; *Statistically insignificant trend $p \geq 0.05$

Supplementary Figure 1. Survival time of conversion to dementia. Non-converters MCI (n=3) were censored (15%).



Supplementary Figure 2. Survival time of conversion to dementia. Mean of covariates.



Artículo III

6.3. Genome-wide significant risk factors for Alzheimer's disease: role in progression to dementia due to Alzheimer's disease among subjects with mild cognitive impairment.

Lacour A*, Espinosa A*, Louwersheimer E, Heilmann S, Hernández I, Wolfsgruber S, Fernández V, Wagner H, Rosende-Roca M, Mauleón A, Moreno-Grau S, Vargas L, Pijnenburg YA, Koene T, Rodríguez-Gómez O, Ortega G, Ruiz S, Holstege H, Sotolongo-Grau O, Kornhuber J, Peters O, Frölich L, Hüll M, Rüther E, Wiltfang J, Scherer M, Riedel-Heller S, Alegret M, Nöthen MM, Scheltens P, Wagner M, Tárraga L, Jessen F, Boada M, Maier W, van der Flier WM, Becker T*, Ramirez A*, Ruiz A*.

*Contributed equally.

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ORIGINAL ARTICLE

Genome-wide significant risk factors for Alzheimer's disease: role in progression to dementia due to Alzheimer's disease among subjects with mild cognitive impairment

A Lacour[1,18], A Espinosa[2,18], E Louwersheimer[3], S Heilmann[4,5], I Hernández[2], S Wolfsgruber[1,6], V Fernández[2], H Wagner[6], M Rosende-Roca[2], A Mauleón[2], S Moreno-Grau[2], L Vargas[2], YAL Pijnenburg[3], T Koene[3], O Rodríguez-Gómez[2], G Ortega[2], S Ruiz[2], H Holstege[7], O Sotolongo-Grau[2], J Kornhuber[8], O Peters[9], L Frölich[10], M Hüll[11], E Rüther[12], J Wiltfang[12], M Scherer[13], S Riedel-Heller[14], M Alegret[2], MM Nöthen[4,5], P Scheltens[3], M Wagner[1,6], L Tárraga[2], F Jessen[1,6,15], M Boada[2], W Maier[1,6], WM van der Flier[3,16], T Becker[1,17,18], A Ramirez[4,6,18] and A Ruiz[2,18]

Few data are available concerning the role of risk markers for Alzheimer's disease (AD) in progression to AD dementia among subjects with mild cognitive impairment (MCI). We therefore investigated the role of well-known AD-associated single-nucleotide polymorphism (SNP) in the progression from MCI to AD dementia. Four independent MCI data sets were included in the analysis: (a) the German study on Aging, Cognition and Dementia in primary care patients ($n = 853$); (b) the German Dementia Competence Network ($n = 812$); (c) the Fundació ACE from Barcelona, Spain ($n = 1245$); and (d) the MCI data set of the Amsterdam Dementia Cohort ($n = 306$). The effects of single markers and combined polygenic scores were measured using Cox proportional hazards models and meta-analyses. The clusterin (*CLU*) locus was an independent genetic risk factor for MCI to AD progression (*CLU* rs9331888: hazard ratio (HR) = 1.187 (1.054–1.32); $P = 0.0035$). A polygenic score (PGS1) comprising nine established genome-wide AD risk loci predicted a small effect on the risk of MCI to AD progression in *APOE*-ε4 (apolipoprotein E-ε4) carriers (HR = 1.746 (1.029–2.965); $P = 0.038$). The novel AD loci reported by the International Genomics of Alzheimer's Project were not implicated in MCI to AD dementia progression. SNP-based polygenic risk scores comprising currently available AD genetic markers did not predict MCI to AD progression. We conclude that SNPs in *CLU* are potential markers for MCI to AD progression.

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INTRODUCTION

Alzheimer's disease (AD) is the most common form of neurodegenerative dementia, representing 50–60% of all dementia cases. AD pathology commences years, or even decades, before the appearance of clinical symptoms, and current consensus among scientists is that prevention should be started at an early phase in individuals at increased risk. Patients with mild cognitive impairment (MCI) are at increased risk of developing AD dementia. However, the MCI group is heterogeneous, and wide variation in the annual progression to AD dementia rate has been reported, with estimates ranging from 4 to 31%. In a recent study, which involved the follow-up of 550 MCI subjects for an average of 26.6 months,[1] the present authors found that the majority (45.5%) of those MCI individuals who subsequently developed dementia displayed the AD dementia phenotype. Thus, predicting which MCI cases will actually progress to AD dementia is an important challenge. Several clinical measures and biomarkers have been proposed for this purpose, including neuroimaging, cerebrospinal levels of amyloid-β and phosphorylated and total tau. However, the predictive value of these biomarkers is low.[2,3] Accordingly, research conducted in recent decades has tended to focus on identifying factors that render MCI patients more susceptible to AD dementia.[4] This research is important as the early detection of AD will be essential once an efficacious method of preventing or delaying the disease becomes available.

Individual risk for AD is determined by genetic, environmental and demographic factors, as well as interactions between them. The estimated genetic component of AD, that is, the so-called

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heritability, is as high as 79%. Hence in AD, the majority of pathophysiological pathways are likely to be driven by, or include, genetic determinants. Recent genome-wide association studies (GWAS) and whole-exam sequencing approaches have indeed identified several common and rare low-penetrance risk variants.[5–16]

Within routine clinical practice, the implementation and evaluation of AD risk markers in the prediction of MCI to AD dementia progression is in its inception. To date, the *APOE* (apolipoprotein E) locus is the only marker to have shown a consistent association with MCI to AD progression.[17] For other reported AD genetic markers, studies of MCI to AD dementia progression using single-nucleotide polymorphisms (SNPs), or combinations of SNPs in polygenic scores (PGS), have generated conflicting results.[18,19]

The aim of the present study was to investigate the role of established AD genetic markers in the progression of MCI to AD using follow-up data from four independent MCI data sets ($n = 3216$ subjects).

MATERIALS AND METHODS

Patients

The present cohort comprised MCI patients from Germany, Spain and the Netherlands. These individuals were drawn from the following cohorts: (a) the German study on Aging, Cognition and Dementia in primary care patients (AgeCoDe; $n = 853$);[20] (b) the German Dementia Competence Network (DCN; $n = 812$);[21] (c) the Fundació ACE from Barcelona (ACE, $n = 1245$);[1] and (d) the MCI data sets of the Amsterdam Dementia Cohort (ADC, $n = 306$).[22] Effective sample size varied depending on phenotype analyses and covariation matrices (Table 1). Clinical characteristics, neuropsychological assessment, behavioral and functional scales, and progression to AD dementia rates for each MCI data set are shown in Table 1, Supplementary Table 1 and at http://detritus.fundacioace.com/pgs. The study was approved by the respective ethics committees, and all participants provided written informed consent before inclusion.

DNA extraction, SNP selection and genotyping

DNA from 3216 MCI samples was extracted using commercial methods. SNP selection was based on a review of the literature. Here, only those SNPs in loci identified by GWAS or meta-GWAS efforts were selected. To avoid missing loci, for all of the loci selected for PGS construction, whenever possible, alternative SNPs in linkage disequilibrium were also selected (i.e. linkage disequilibrium proxies). This additional SNP thus served as a backup in the event that the primary selected SNP failed in the sequenom assay. Further details on the references used to select SNPs, the genotyping procedures and genotyping quality control are provided in Supplementary Table 2 and in the *Genotyping procedures* section of the Supplementary Data file. The sequenom technology genotyping methods are described elsewhere.[16]

Statistical analysis

To investigate the influence of genetic markers, demographic factors and PGS on MCI to AD dementia progression, methods from survival analysis were used. For the 40 individual SNPs and the three PGS of interest, hazard ratios (HRs) were calculated using the following three models: (i) crude (model 0); (ii) age- and gender-adjusted (model 1); and (iii) age-, gender-, *APOE*- and education-adjusted (model 2) (for details see Statistical Analysis in the Supplementary Data file). Unless otherwise specified, the subsequent text refers to model 1 only.

PGS construction and evaluation

PGS were calculated in accordance with Purcell *et al.*[23] (for details, see Polygenic Score Construction and Polygenic Score Evaluation in the Supplementary Data file). PGS were constructed using sets of AD-associated loci identified in recent GWAS. Inclusion of SNPs in the PGS was based on definitive evidence of association in large meta-GWAS reported by the International Genomics of Alzheimer's project (IGAP).[15] Since the established association between *APOE* ε4 and AD is also present in our four cohorts, the *APOE* region was excluded from the PGS calculation. PGS1 comprised the nine established AD-associated SNPs reported before publication of the IGAP consortium results (see Supplementary Table 3 and Part A in the Supplementary Data file). PGS2 comprised 9 of the 11 novel AD-associated SNPs identified by IGAP (Supplementary Table 3 and Part B in the Supplementary Data file).[15] PGS3 comprised all SNPs from PGS1 and 2. Each of the three calculated PGS was used as a dose, and the proportional hazards model was employed using the three models applied for the analysis of single SNPs. Meta-analysis techniques were used to estimate the global effects of SNPs and PGS. The meta-analysis was conducted using the standard fixed effect approach implemented in the YAMAS software. YAMAS implements standard fixed

Table 1. Effective sample size and baseline demographics in data sets

	AgeCoDe	*DCN*	*ACE*[a]	*ADC*
Subjects	853	812	1245	316
Detected duplicities	−1	−7	0	0
Detected non-MCI subjects	−46	0	0	0
No/low genotypes	−3	−6	0	−10
No follow-up data[b]	−299	−201	−74	0
No age/sex data	0	−1	0	0
Effective sample size (model 1)	504	597	1171	306
No *APOE*/education data	−4	−157	−1	−23
Effective sample size (model 2)	500	440	1170	283
AD converters (model 1)	209 (41.4%)	76 (12.7%)	395 (33.7%)	110 (35.9%)
AD converters (model 2)	207 (41.4%)	73 (16.5%)	395 (33.8%)	100 (35.3%)
Age (years) (mean)	81.6	66.2	76.0	66.8
Age (years) (s.d.)	4.1	8.9	7.1	7.8
Follow-up time (months) (mean)	43.0	26.9	26.0	27.7
Observational time (months) (s.d.)	25.4	11.2	18.9	17.7
Time to conversion (mean)	38.6	19.2	21.1	27.1
Time to conversion (s.d.)	22.6	8.5	16.0	17.4
Gender (%), female	69.4	43.7	64.6	38.9
APOE-ε4 (%)	25.8	34.2	32.4	52.3
Education % of high education (>3 points in harmonized scores)	13.6	5.6	8.1	15

Abbreviations: ACE, The Fundació ACE from Barcelona; AD, Alzheimer's disease; ADC, Amsterdam Dementia Cohort; AgeCoDe, German study on Aging, Cognition and Dementia in primary care patients; *APOE*, apolipoprotein E; DCN, German Dementia Competence Network; MCI, mild cognitive impairment. [a]ACE, $n = 1245$. [b]Subjects genotyped but without follow-up.

and random-effects meta-analysis, and operates on beta and standard error.[24]

RESULTS

Univariate analyses

The demographic characteristics of the cohorts are summarized in Table 1. The results obtained for each analyzed SNP are shown in Table 2.

In the meta-analysis, the *APOE*-ε4 allele (rs429358 C allele) showed an association with the rate of MCI to AD dementia progression in all cohorts, with a homogeneous effect being observed across data sets (HR = 1.84 (1.64–2.04), heterogeneity index (I^2) = 0, $P = 1.35 \times 10^{-27}$) (Figures 1a and 2a). Interestingly, the relative risk was ~50% of that reported in GWAS.[6–9] Furthermore, the ε4 effect increased with age, reaching its most pronounced effect between 65 and 80 years. In contrast, the *APOE*-ε2 allele conferred a protective effect against MCI to AD dementia progression to other *APOE* genotypes (Figure 1a). As with ε4, the effect of *APOE*-ε2 was dose dependent and homogeneous across data sets. The meta-analysis confirmed the protective effect of *APOE*-ε2 (HR = 0.69 (0.51–0.86), $I^2 = 0$, $P = 0.004$; Table 2). Six MCI subjects carrying the *APOE*-ε2 allele in a homozygous state did not progress to AD dementia during the observational time period.

An additional association signal was observed in SNPs at the *CLU* locus (rs9331888, rs11136000). For these variants, a nominally significant result was obtained in the AgeCoDe cohort, and a consistent trend towards association was observed in the DCN, ACE and ADC cohorts (Table 2). The meta-analysis yielded a significant association for both *CLU* SNPs ($P = 0.003$ and 0.01, respectively). Although rs11136000 showed a heterogeneous HR across the series, the HR for rs9331888 was homogeneous across the four cohorts (Table 2). Association findings for the *CLU* SNPs withstood all adjustments (Table 2, Figure 1b and Supplementary Data files). No major difference in the effect sizes of the *CLU* SNPs was observed following stratification for *APOE* status, gender or age (Table 4 and Figure 2b; $P > 0.71$). Stratification for these variables confirmed the orthogonality of *CLU* markers with key covariates.

Of the remaining SNPs genotyped in the present study, only rs641120 (located at the *SORL1* locus) showed nominal significance with MCI to AD dementia progression (HR = 0.89, $P = 0.043$, model 0). However, this finding did not withstand adjustment.

PGS in MCI to AD dementia progression

The results of the hazard models analysis of PGS are shown in Table 3. In the meta-analysis of PGS1, a trend towards association was observed (HR = 1.31, $P = 0.1$). Interestingly, stratification according to *APOE* genotype revealed a consistently higher effect size for PGS1 in *APOE*-ε4 carriers (Table 4). The meta-analysis of PGS1 showed that the effect in *APOE*-ε4 carriers was nominally significant (HR = 1.74 (1.03–2.97), $P = 0.04$). However, combined analysis revealed no statistically significant interaction between PGS1 and the *APOE* locus in any of the four data sets ($P = 0.14$). In contrast, PGS2 did not contribute to MCI to AD dementia progression. The effect size for PGS2 observed in the meta-analyses indicated a nonsignificant protective effect. This suggests that the accumulation of risk alleles was implicated in protection from MCI to AD dementia progression in the present series.

The analysis of PGS3 yielded an intermediate and nonsignificant result (HR = 1.03, $P = 0.96$). The PGS3 results reflect the findings of PGS1, as biased by the noise from PGS2. No significant interaction was found between PGS3 and age, gender, *APOE*-ε4 status or cohort (Tables 3 and 4).

DISCUSSION

For several years, intensive research has attempted to identify the role of genetic factors in the progression of MCI to AD dementia. To date, however, only the *APOE* locus has shown a consistent association. Elias-Sonnenschein *et al.*[17] performed a meta-analysis of 35 prospective MCI studies, which comprised a total of 6095 subjects. Of these, 1236 individuals progressed to AD dementia within a 2.9-year period of follow-up. For MCI subjects carrying the *APOE*-ε4 allele, the authors reported an odds ratio of 2.29 (1.88–2.80) for progression to AD dementia. The present findings support the hypothesis that the *APOE*-ε4 allele is implicated in MCI to AD dementia progression (HR = 2.20 (1.88–2.53) for subjects carrying *APOE-ε4* allele). However, we cannot exclude the possibility that additional loci around *APOE* may also modulate the age at onset for AD, as has been suggested for *TOMM40*, a gene adjacent to *APOE*.[25,26] Detailed mapping data of the linkage disequilibrium region around *APOE* are now available. These have identified a poly-T length polymorphism in an intron of *TOMM40*. Interestingly, research has demonstrated that the allele distribution of the poly-T polymorphism explains a larger proportion of the observed survival curves of age at onset in AD than is the case for *APOE*-ε4 containing haplotypes alone.[25,26] To confirm the role of *TOMM40* poly-T in AD progression, genotyping of this poly-T is currently being scheduled in our large MCI data set.

In the present study, the MCI to AD dementia progression rate increased continuously with age, whereas the effect of the allele *APOE*-ε4 on AD dementia progression decreased after the age of 80 years (Figure 2a). However, previous research has shown that both the incidence of AD and the AD risk effect of *APOE*-ε4 decrease in the elderly.[27,28] The observation of a reduced association between *APOE*-ε4 and MCI to AD dementia progression is consistent with the survivor effect, as *APOE*-ε4 is a risk factor for both a shorter lifespan and dementia.[29] A plausible hypothesis therefore is that most *APOE*-ε4-carrying MCI patients from the present cohorts had converted to dementia or died at an earlier age, thereby causing an enrichment of survivor *APOE*-ε4 MCI carriers among our elderly MCI subjects. This latter group is protected against the progression risk effect conferred by *APOE*-ε4, and this may have led to the observed reduction in the association between *APOE*-ε4 and progression to AD dementia in the present study. This hypothesis is supported by the fact that a reduced *APOE*-ε4 allele frequency was found within this age group compared with younger individuals (Figure 2a).

Besides *APOE*, no other SNP or PGS combination reached study-wise statistical significance (Bonferroni-corrected *P*-value = 0.00125). However, for some of these markers (i.e. SNPs contributing to PGS1), definitive evidence of association AD has been reported. Hence, the application of Bonferroni correction in this context could be considered overconservative, as our study was based on validated AD susceptibility loci.

The univariate analyses identified a consistent effect on MCI to AD dementia progression for two SNPs (rs11136000 and rs9331888) in the *CLU* gene ($P = 0.0035$). For both SNPs, a small but consistent effect was observed in all four series, as well as in the meta-analysis. The effect sizes and allele directions of both SNPs are consistent with those reported in previous AD case control GWAS.[7] Rodriguez-Rodriguez *et al.*[19] also obtained a significant result for rs11136000 allele T in MCI to AD dementia progression in a small data set. The effect size observed in the Rodriguez-Rodriguez series[9] was inflated compared with both the present data and previous results on the role of *CLU* markers in AD risk.[6,7,15] Nonetheless, the reported confidence interval overlaps with our evaluation. Therefore, the present *CLU* results represent an independent replication of a previous report, and have confirmed, in a much larger sample size, the involvement of *CLU* in MCI to AD dementia progression.

Table 2. Effect of candidate SNPs on conversion of mild cognitive impairment to Alzheimer's disease[a]

Gene	*SNP*	*Chr.*	*Position*	*Minor/major2 meta-analysis*				*AgeCoDe sample*				*DCN sample*			*ACE sample*			*ADC sample*		
				Allele	P-*value*	*HR*	σ*HR*	I^2	P-*value*	*HR*	σ*HR*	P-*value*	*HR*	σ*HR*	P-*value*	*HR*	σ*HR*	P-*value*	*HR*	σ*HR*
ABCA7	Rs3764650	19	1 046 520	G/T	0.2350	0.90	0.08	0.0	0.8280	0.96	0.17	0.3147	0.72	0.23	0.6034	0.94	0.11	0.2485	0.76	0.18
ABCA7	Rs3752246	19	1 056 492	G/C	0.2265	0.90	0.08	27.5	0.4360	0.90	0.12	0.0799	0.64	0.16	0.7572	1.03	0.10	0.1996	0.79	0.15
ADAMST20	Rs7295246	12	43 967 677	G/T	0.4310	1.04	0.05	0.0	0.8770	0.99	0.09	0.2523	1.20	0.19	0.7416	1.02	0.07	0.4502	1.11	0.15
BIN1	Rs7561528	2	127 889 637	A/G	0.5507	1.03	0.06	0.0	0.7590	0.97	0.10	0.1905	1.25	0.22	0.9193	1.01	0.09	0.4831	1.11	0.16
BIN1	Rs744373	2	127 894 615	C/T	0.4857	1.04	0.06	4.0	0.7340	1.04	0.11	0.1277	1.31	0.23	0.5729	0.95	0.09	0.3606	1.15	0.18
CASS4	Rs7274581	20	55 018 260	C/T	0.6657	0.96	0.08	0.0	0.9540	1.01	0.17	0.3610	1.27	0.33	0.4805	0.92	0.11	0.4553	0.82	0.21
CD2AP	Rs10948363	6	47 487 762	G/A	0.6454	0.97	0.06	0.0	0.5560	0.93	0.11	0.9713	0.99	0.18	0.7333	0.97	0.09	0.8185	1.04	0.16
CD33	Rs3865444	19	51 727 962	A/G	0.3575	1.05	0.06	0.0	0.4270	1.09	0.11	0.3488	1.17	0.19	0.9048	0.99	0.09	0.5796	1.08	0.15
CLU	Rs11136000	8	27 464 519	T/C	0.0111	0.87	0.05	0.0	0.0184	0.78	0.08	0.2912	0.84	0.14	0.1411	0.89	0.07	0.8962	1.02	0.15
CLU	Rs9331888	8	27 468 862	C/G	0.0035	1.19	0.07	0.0	0.1380	1.17	0.13	0.7383	1.06	0.18	0.0975	1.16	0.10	0.0210	1.41	0.21
CR1	Rs6656401	1	207 692 049	A/G	0.6741	0.95	0.11	58.1	0.6520	0.95	0.12	0.1433	0.72	0.16	0.0560	1.21	0.12	0.2749	0.82	0.15
CR1	Rs3818361	1	207 784 968	C/T	0.9333	0.99	0.08	19.3	0.6020	0.94	0.12	0.2388	0.78	0.16	0.1693	1.15	0.12	0.6728	0.93	0.16
ECHDC3	Rs7920721	10	11 720 308	G/A	0.4273	1.04	0.05	0.0	0.5920	1.06	0.11	0.4970	0.89	0.15	0.5159	1.05	0.08	0.4677	1.11	0.16
EPHA1	Rs10808026	7	143 099 133	A/C	0.7468	0.98	0.06	0.0	0.7650	0.97	0.11	0.9316	0.98	0.21	0.9940	1.00	0.10	0.7267	0.94	0.17
FERMT2	Rs17125944	14	53 400 629	C/T	0.3620	0.92	0.09	0.0	0.1420	0.77	0.14	0.9712	0.99	0.30	0.9970	1.00	0.15	0.7627	0.94	0.18
FRMD4A	Rs7081208	10	13 991 865	A/G	0.2568	0.90	0.09	53.2	0.3570	1.11	0.12	0.0276	0.65	0.13	0.3280	0.90	0.09	0.2491	0.84	0.12
FRMD4A	Rs17314229	10	14 016 159	T/C	0.7526	1.04	0.11	0.0	0.6330	0.91	0.18	0.9726	0.99	0.30	0.9779	1.00	0.18	0.1650	1.44	0.38
GAB2	Rs2373115	11	78 091 150	T/G	0.5383	0.96	0.07	0.0	0.4200	0.90	0.12	0.4543	0.84	0.20	0.8222	1.02	0.11	0.8089	0.96	0.17
HS3ST1	Rs6448799	4	11 630 049	T/C	0.5308	0.96	0.06	20.1	0.3880	1.10	0.12	0.3730	0.85	0.15	0.0933	0.88	0.07	0.6739	1.07	0.18
INPP5D	Rs35349669	2	234 068 476	T/C	0.9073	1.01	0.07	29.9	0.5260	1.06	0.10	0.2404	0.82	0.14	0.5088	0.95	0.07	0.1509	1.23	0.18
MEF2C	Rs190982	5	88 223 420	G/A	0.1918	1.10	0.08	44.2	0.6150	0.95	0.10	0.4999	1.12	0.19	0.0018	1.26	0.09	0.7074	1.06	0.15
MS4A	Rs4938933	11	60 034 429	C/T	0.3051	0.93	0.06	26.9	0.6660	1.04	0.11	0.6523	1.08	0.18	0.0230	0.83	0.07	0.4229	0.89	0.13
MTHFD1L	Rs11754661	6	151 207 078	A/G	0.8502	0.98	0.11	0.0	0.8420	1.05	0.24	0.9704	0.99	0.29	0.2979	0.84	0.14	0.2580	1.38	0.40
NDUFAF6	Rs7818382	8	96 054 000	T/C	0.1804	1.07	0.05	0.0	0.5010	1.07	0.11	0.5276	1.10	0.16	0.4346	1.06	0.07	0.5178	1.09	0.15
NME8	Rs2718058	7	37 841 534	G/A	0.3797	1.09	0.11	69.0	0.6790	0.96	0.09	0.0196	1.49	0.25	0.2620	0.92	0.07	0.0901	1.29	0.19
None	Rs6678275	1	193 625 233	C/G	0.9538	1.00	0.06	0.0	0.3890	1.11	0.14	0.9359	0.98	0.20	0.2446	0.90	0.09	0.4404	1.13	0.18
PICALM	Rs561655	11	85 800 279	G/A	0.3934	0.95	0.05	0.0	0.3840	0.91	0.10	0.4566	1.13	0.19	0.6238	0.96	0.08	0.4172	0.89	0.13
PICALM	Rs3851179	11	85 868 640	A/G	0.5097	0.96	0.05	0.0	0.8660	0.98	0.10	0.5659	1.10	0.19	0.6135	0.96	0.08	0.2728	0.85	0.13
PILRA	Rs2405442	7	99 971 313	A/G	0.6871	0.97	0.07	27.0	0.2180	0.87	0.10	0.1933	0.79	0.14	0.6401	1.04	0.08	0.3719	1.14	0.16
PILRA	Rs34995835	7	99 990 364	T/G	0.6823	0.98	0.06	0.0	0.3520	0.90	0.10	0.3331	0.84	0.15	0.7368	1.03	0.08	0.7445	1.05	0.16
PTK2B	Rs28834970	8	27 195 121	C/T	0.9757	1.00	0.06	8.6	0.8990	1.01	0.11	0.1338	0.77	0.13	0.9222	1.01	0.07	0.3142	1.16	0.17
SCIMP	Rs7225151	17	5 137 047	A/G	0.1055	1.13	0.08	0.0	0.9920	1.00	0.15	0.9477	0.98	0.25	0.0813	1.19	0.12	0.3362	1.23	0.27
SLC24A4	Rs10498633	14	92 926 952	T/G	0.3628	0.89	0.11	64.5	0.4030	1.10	0.13	0.1515	0.73	0.16	0.7479	1.03	0.10	0.0151	0.62	0.12
SORL1	Rs641120	11	121 380 965	T/C	0.0774	0.91	0.05	0.0	0.3140	0.90	0.09	0.9097	0.98	0.16	0.1913	0.90	0.07	0.4198	0.89	0.13
SORL1	Rs11218343	11	121 435 587	C/T	0.9564	0.99	0.17	37.0	0.2380	1.30	0.29	0.3904	1.31	0.41	0.2513	0.78	0.17	0.2515	0.64	0.25
SORL1	Rs2070045	11	121 448 090	G/T	0.7424	0.98	0.06	0.0	0.6830	0.95	0.11	0.9858	1.00	0.20	0.9881	1.00	0.09	0.7583	0.95	0.15
SPPL2A	Rs8035452	15	51 040 798	C/T	0.6115	0.97	0.06	27.1	0.7820	1.03	0.10	0.2125	1.23	0.20	0.1252	0.89	0.07	0.3613	0.87	0.13
TOMM40	Rs2075650	19	45 395 619	G/A	1.19e − 14	1.62	0.10	0.0	1.02e − 04	1.56	0.18	0.0032	1.67	0.29	1.53e −07	1.76	0.19	0.0022	1.49	0.19
TREML2	Rs9381040	6	41 154 650	T/C	0.7648	0.98	0.08	40.6	0.9700	1.00	0.11	0.0512	0.70	0.13	0.2735	1.09	0.08	0.7930	0.96	0.14
CWPW1	Rs1476679	7	100 004 446	C/T	0.7958	0.99	0.06	0.0	0.4000	0.91	0.10	0.4148	0.86	0.16	0.7532	1.03	0.08	0.6037	1.08	0.16

Abbreviations: ACE, the Fundacio ACE from Barcelona; ADC, Amsterdam Dementia Cohort; AgeCoDe, German study on Aging, Cognition and Dementia in primary care patients; Chr., chromosome; DCN, German Dementia Competence Network; HR, hazard ratio; σHR, hazard ratio standard deviation; I^2, heterogeneity index; SNP, single-nucleotide polymorphism. [a]HRs were calculated with univariate Cox proportional hazard model with adjustment for age and gender (model 1).

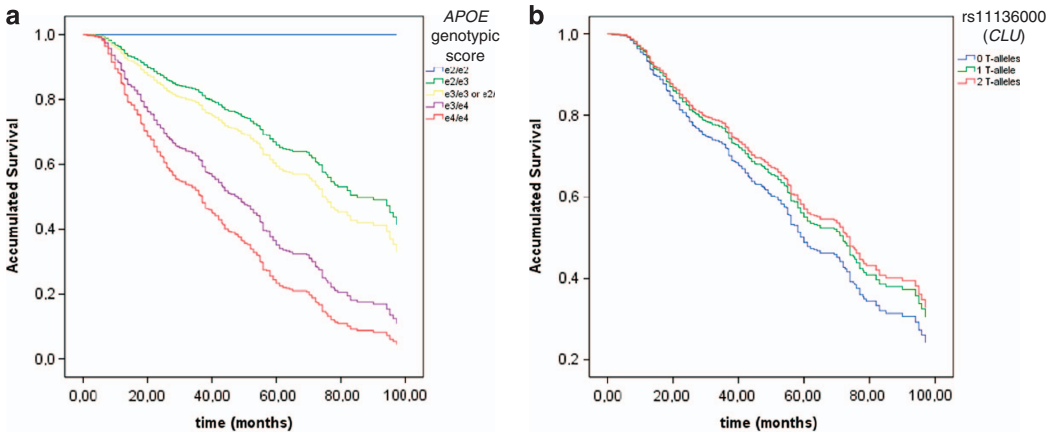

Figure 1. Cox proportional hazard model multivariate dementia-free survival analyses for *APOE* (apolipoprotein E) genotypic score (**a**) and clusterin (*CLU*) rs111360000 (**b**). Hazard ratio meta-analyses were adjusted according to data set, age and gender.

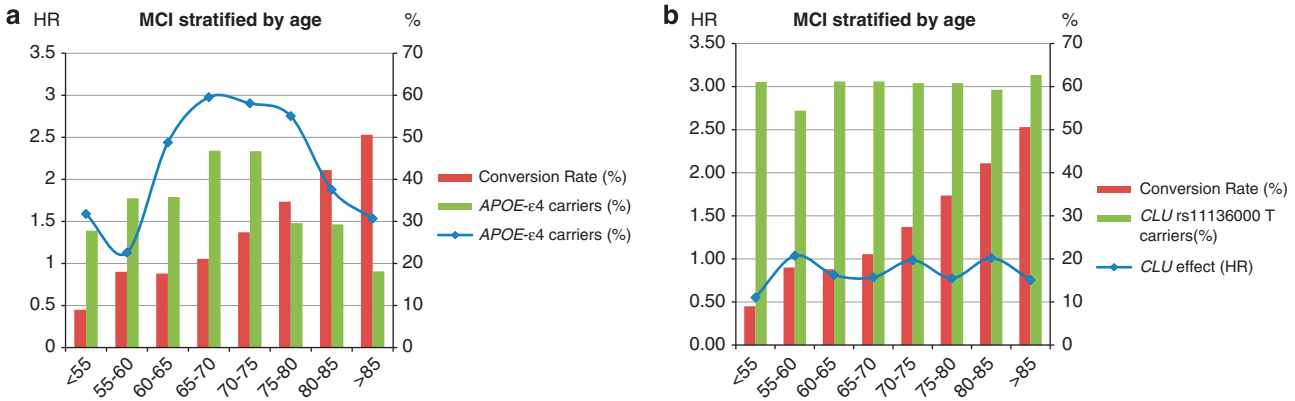

Figure 2. Effect size of the *APOE* (apolipoprotein E) (**a**) and clusterin (*CLU*) (**b**) loci in mild cognitive impairment (MCI) to Alzheimer's disease (AD) dementia progression following stratification for age. *Notes*: Meta-analysis of hazard ratio (HR) for progression to AD dementia in *APOE*-ε4 carriers following stratification for age. The progression rate for each age stratum is shown in the secondary Y2 axis.

Interestingly, other research has shown that the rate of cognitive decline among individuals who were cognitively normal at study baseline, but who subsequently developed MCI or AD, was significantly faster in those carrying the C allele of rs11136000 compared with non-carriers.[30] Furthermore, cognitively normal carriers of the risk allele C of rs11136000 have been reported to show a significant increase in regional cerebral blood flow in brain areas intrinsic to memory processes.[30] Overall, the genetic evidence supports the hypothesis that the *CLU* locus makes an independent contribution to MCI to AD dementia progression.

Along the same lines, the gene product of the *CLU* gene, clusterin/apolipoprotein J, has been proposed as a potential biomarker for AD. In this regard, the plasma concentration of apolipoprotein J has been associated with the severity and speed of disease progression in AD patients, as well as with atrophy of the entorhinal cortex and the hippocampus in AD.[31,32] In the prodromal stages of AD, for example, MCI elevated plasma levels of apoliprotein J have also been associated with lower rate of brain atrophy.[33] This atrophy involved the hippocampus and the entorhinal cortex, that is, brain regions affected in the early stages of AD pathogenesis. Together, these findings suggest that clusterin levels respond in a selective manner along the cascade of events occurring in AD, and that this commences during the prodromal stages. This protective plasma response may modulate, at least in part, the progression of MCI to AD dementia. Our data provide additional support for this hypothesis, as they demonstrate an association between genetic variability in *CLU* and MCI to AD dementia progression. Although the precise molecular mechanism through which genetic variability in *CLU* modulates plasma clusterin levels remains unclear, research suggests the potential involvement of genetic variability in *CLU* in the modulation of gene expression. Hence, *CLU* appears a promising therapeutic target for AD.

The lack of association for most of the investigated SNPs in the present study may suggest that AD susceptibility loci have only small effects in terms of MCI to AD dementia progression risk. If this is the case, the present MCI data sets would have limited statistical power to detect them. Another power-reducing factor may have been the inclusion in MCI subjects who will never develop AD dementia or who will convert to other unrelated forms of dementia. In support of this, the effect sizes of true AD susceptibility genes in the present MCI series were low compared with conventional AD case–control data sets (OR = 3.5 vs HR = 2.2 for *APOE*), and the progression rate for elderly MCI subjects was higher compared with that in the case–control context. An alternative explanation is that the relative risk in GWAS studies was obtained from analysis of progression from healthy control status to AD dementia. In this case, only part of this relative risk was examined in the present study, as our series comprised individuals who were already diagnosed with MCI, many of whom

Table 3. Effect of PGS on conversion from mild cognitive impairment to Alzheimer's disease[a]

PGS	*Meta-analysis*				*AgeCoDe sample*			*DCN sample*			*ACE sample*			*ADC sample*		
	P-*value*	*HR*	*95% CI*	I^2	P-*value*	*HR*	*95% CI*	P-*value*	*HR*	*95% CI*	P-*value*	*HR*	*95% CI*	P-*value*	*HR*	*95% CI*
PGS1	0.139	1.29	(0.86; 1.72)	0.0	0.678	1.15	(0.57; 2.21)	0.504	0.70	(0.25; 1.99)	0.041	1.64	(1.02; 2.44)	0.92	1.05	(0.43; 2.56)
PGS2	0.669	0.89	(0.41; 1.37)	33.1	0.690	0.85	(0.40; 1.84)	0.100	0.34	(0.09; 1.23)	0.667	0.88	(0.47; 1.66)	0.201	2.02	(0.68; 6.00)
PGS3	0.625	1.18	(0.37; 2.00)	31.6	0.895	1.07	(0.40; 2.88)	0.130	0.27	(0.05; 1.46)	0.136	1.81	(0.83; 3.94)	0.489	1.66	(0.40; 6.92)

Abbreviations: ACE, the Fundació ACE from Barcelona; AgeCoDe, German study on Aging, Cognition, and Dementia in primary care patients; CI, confidence interval; DCN, German Dementia Competence; Network; HR, hazard ratio; I^2, heterogeneity index; PGS, polygenic score. [a]HRs were calculated in a univariate Cox proportional hazard model with adjustment for age and gender (model 1).

Table 4. Stratification analysis of candidate SNPs or PGS by the presence of *APOE*-ε4 allele

Marker or SNP polygenic score	*APOE-ε4 carriers HR (95% CI);* P-*value*	*APOE-ε4 non-carriers HR (95% CI);* P-*value*	*Overall HR (95% CI);* P-*value*
CLU rs9331888	1.206 (0.95–1.46); $P=0.081$	1.138 (0.96–1.32); $P=0.112$	1.187 (1.054–1.32); $P=0.0035$
PGS1	1.746 (1.029–2.965); $P=0.038$	1.026 (0.650–1.620); $P=0.912$	1.288 (0.86–1.72); $P=0.139$
PGS2 (new IGAP loci)	0.943 (0.496–1.790); $P=0.857$	0.790 (0.441–1.417); $P=0.428$	0.889 (0.41–1.37); $P=0.668$
PGS3 (all loci)	1.824 (0.805–4.132); $P=0.149$	0.903 (0.433–1.883); $P=0.785$	1.186 (0.37–2.00); $P=0.625$

Abbreviations: AD, Alzheimer's disease; *APOE*, apolipoprotein E; CI, confidence interval; HR, hazard ratio; IGAP, International Genomics of Alzheimer's project; PGS, polygenic scores; SNP, single-nucleotide polymorphism. *Note*: Effect sizes were calculated using Cox proportional models adjusted by cohort, age and gender. *CLU* rs933188 effect size was calculated per each T allele. HRs for PGSs were calculated per each score point. PGS1 comprises nine genome-wide significant AD loci reported in advance of IGAP. PGS2 comprised nine confirmed loci reported by IGAP initiative (Lambert *et al.*[15]). PGS3 included 18 genome-wide loci for AD (PGS1+PGS2). For details on PGS construction see Supplementary Table S2 and Materials and methods.

had not yet converted to AD dementia, yet but would do so in the near future.[1] Hence, 'missing' relative risk in MCI studies may be found when analyzing progression of healthy individuals to MCI or by extending the period of follow-up. Alternatively, the lack of association observed for most SNPs in the present study may suggest that many genuine genetic risk factors for AD exert their pathological effects earlier, that is, during pathological processes that occur during the pre-MCI stages of the disease. This hypothesis would imply that differing genetic factors contribute to AD susceptibility as compared with AD progression. In fact, selecting intermediate phenotypes such as MCI, which are more proximal to a specific event along the causal chain of AD, may capture more variations in the underlying heritable traits and further enhance the statistical power of the study. Interestingly, a number of previous studies selected intermediate AD phenotypes for genetic association analyses, which included neuropsychiatric test measures,[34] magnetic resonance imaging data,[35,36] biomarkers from blood and cerebrospinal fluid[37,38] and direct measurements of AD pathology.[39] Most of the association signals identified by these studies do not overlap with known genetic susceptibility genes.

On the other hand, genetic studies based on longitudinal samples provide new insight into the pathways related to disease progression. A recent GWAS (based on ^{18}F-florbetapir PET) of time-dependent amyloid accumulation in AD implicated the microglial activation-associated gene, *IL1RAP*.[40] Furthermore, the authors also found that *APOE* and *CLU* affect amyloid accumulation, which is consistent with the known effects of these molecules on disease susceptibility. The *IL1RAP* gene was also associated with a greater likelihood of progression from MCI to AD dementia. Interestingly, the interleukin-1 proinflammatory pathway, to which *IL1RAP* belongs, is involved in plaque-associated activation of microglia and amyloid burden.[41] This inflammatory pathway is shared with *CLU* because clusterin modulates neuroinflammation by inhibiting the inflammatory response associated with complement activation.[42] In the case of *APOE*, research has linked the gene product, apoE, to innate inflammatory responses induced via the *TLR4* and interleukin-4 R-receptor pathways.[43] Furthermore, apoE and clusterin cooperate to regulate the clearance and deposition of amyloid-β in the brain.[44] Notably, clusterin and apoE promote the clearance of amyloid-β by interacting with several receptors located on microglia cells, including *TREM2*.[42,45,46] These findings, together with our own, suggest that immune responses and microglial clearance of amyloid-β have a role in disease progression from MCI to AD dementia. Interestingly, a recent study on AD identified a significant association with signals in SNPs located in genes involved in immune response pathways,[47] suggesting a partial overlap between disease progression pathways and those that increase susceptibility to AD.

Previous AD studies have investigated the predictive value of PGS constructed using the effects sizes of multiple SNPs. For example, Verhaaren *et al.*[48] constructed a genetic risk score (GRS) that was similar to the present PGS1. By using this GRS in 5171 non-dementia cases from Rotterdam, the authors demonstrated that although the GRS without *APOE* was associated with the development of AD ($P=0.010$), it provided only a marginal improvement in the prediction of AD dementia beyond that provided by age, sex and *APOE* status (area under the curve: 0.8159 vs 0.8148, respectively). Using a similar strategy, Rodriguez-Rodriguez *et al.*[19] used a GRS based on eight non-*APOE* genetic AD risk variants to study its effect on MCI to AD dementia progression, and on rapid progression from MCI to AD dementia. Although the authors observed no association between GRS and progression risk, they found that AD converters harboring six or more risk alleles progressed twice as rapidly to AD compared with individuals with less than six risk alleles. Thus, the present findings for PGS1 are consistent with these previous studies, and support the hypothesis that the first identified AD susceptibility locus has only a limited role in MCI to AD dementia progression. Interestingly, whereas PGS1 achieved nominal significance in *APOE*-ε4 carriers in the present study, this was not observed by Rodriguez-Rodriguez *et al.*[19] (Table 4). This may have been due to an enrichment of truly prodromal AD within *APOE*-ε4 carriers, who were therefore likely to progress to AD dementia within our

observational time. Nevertheless, this observation with PGS1 suggests that AD susceptibility genes other than *APOE* also contribute to disease progression. However, the predictive value of the PGS1 composite effect for diagnosis is too small to improve prediction, and this precludes its use in routine clinical practice.

The markers included in PGS1 are the best AD-associated SNP set reported to date, as—with the exception of *CD33*—all were reconfirmed in the large replication data set included in the IGAP effort. Many of the SNPs discovered by IGAP only reached GWAS significance during the last round of replication. Consequently, many of these SNPs still await an extensive independent replication effort to confirm genuine loci and remove false positives.[16] The existence of some false positives among the IGAP results cannot be excluded, and this would affect PGS results.

The present study had several limitations. First, the sample size may have been too small to detect certain associations. Unexpectedly, we observed a worsening of PGS risk prediction following the addition of the novel SNPs identified by the IGAP. In fact, a nonsignificant protective effect was observed for PGS2 in our meta-analyses ($P = 0.25$). A possible explanation for this finding is that novel loci included in PGS2 have even smaller effect sizes than the SNPs included in PGS1, in which case our sample would have been too small to detect association. Alternatively, the effect of the IGAP-SNPs may be restricted to very late or early onset AD, or to an undetermined and very specific subgroup of AD patients that was poorly represented in our MCI data sets. Second, only 9 of 11 novel risk loci found by IGAP were represented in PGS2 and PGS3. Unfortunately, the genotyping method failed for rs9271192 at *HLA-DRB5–HLA-DRB1*, and for rs10838725 at *CELF1*, and no additional backup SNPs were available for either locus. Thus, the conclusions drawn for PGS2 and PGS3 should be viewed with caution. Notwithstanding, the small effect sizes of the two markers are unlikely to have made a strong contribution to the overall effect of PGS2 or, more particularly, PGS3. Nevertheless, future efforts are necessary to investigate the potential implications of these missing markers (either by themselves or in combination with other loci) in terms of the progression of MCI to AD dementia.

In summary, the present data support the hypothesis that *CLU* has an independent role in MCI to AD progression. As in previous studies, the data also confirm the role of *APOE* in this process. Furthermore, our longitudinal data suggest that the genetic effect of AD risk factors on MCI progression may be age-dependent. Finally, our findings confirm the poor predictive value of the current genome-wide AD risk loci for MCI to AD dementia progression. Further studies in larger longitudinal MCI samples are now warranted to replicate these data, and to disentangle the genetic factors that influence the progression of MCI to AD dementia. Information on loci acting in the prodromal stages of AD, that is, in patients with MCI, will be of relevance for drug target selection in secondary prevention trials.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on the Molecular Psychiatry website (http://www.nature.com/mp)

Supplementary table 1: Neuropsychological assessment, Behavioural and Functional scales, from datasets.

AgeCoDe	DCN	BCN	ADC
SIDAM Orientation in time and place Delayed recall of a word list and a fictitious name and address Delayed visual reproduction Biographical memory questions Abstract reasoning Serial calculation Backward spelling Backward digit span Figures copy Naming objects Reading and obeying a sentence Writing a sentence Performing a three-stage command	**CERAD** Verbal Fluency Visual Naming (15-BNT) Word List Memory Constructional Praxis Word List Recall Word List Recognition Recall of Constructional Praxis FCSRT	**NBACE** Global orientation Verbal Learning WMS-III Delayed recall WMS-III Recognition Memory WMS-III Digit Span Forward WAIS-III Digit Span Backwards WAIS-III Block Design WAIS-III Imitation Praxis Ideomotor Praxis Visual Naming (15-BNT) Poppelreuter's test (responses) 15-OT (responses) Luria's Clock test Automatic Inhibition SKT (time) Automatic Inhibition SKT (error) Phonetic verbal fluency Semantic verbal fluency Similarities WAIS-III	**CAMCOG** VAT Immediate recall RAVLT Delayed recall RAVLT Naming VAT Letter fluency COWAT Digit Span Forward WAIS-III Digit Span Backwards WAIS-III Incomplete letters VOSP Dot counting VOSP Number location VOSP TMT-A TMT-B FAB Phonetic verbal fluency Semantic verbal fluency
MMSE	**MMSE**	**MMSE**	**MMSE**
GDS	**ADAS-Cog**	**GDS**	**GDS**
CDR	**CDR-SB**	**CDR**	**CDR**
SIDAM-ADL-Scale	**ADCS-MCI-ADL**	**BDRS**	**DAD**
BDRS	**NPI**	**NPI-Q**	**NPI**
	MADRS		**Amsterdam IADL-Q**
	RUD		

SIDAM: A structured interview for the diagnosis of dementia of the Alzheimer type, multi-infarct (or vascular) dementia and dementias of other aetiology according to DSM-III-R, DSM-IV, and ICD-10; MMSE: Mini Mental State Examination; GDS: Global Deterioration Scale; SIDAM-ADL-Scale: a 14-item scale for the assessment of activities of daily living; BDRS: Blessed Dementia Rating scale; CERAD: Clinical and Neuropsychology Assessment; 15-BNT: the abbreviated Boston Naming Test with 15 items; FCSRT: Free and Cued Selective Reminding Test; ADAS-Cog: Alzheimer's Disease Assessment Scale-cognitive subscale; CDR-SB: Clinical Dementia rating Sum of Boxes; ADCS-MCI-ADL: Alzheimer's disease Cooperative Study scale for Activities of Daily Living in MCI; NPI: Neuropsychiatric Inventory; MADRS: Montgomery-Asberg depression scale; RUD: Resource Utilization in Dementia Questionnaire Scale; NBACE: Neuropsychological battery of Fundació ACE; Verbal learning WMS-III = 1st+2nd+3rd+4thtrial scores; WMS-III: Wechsler Memory Scale, Third Edition; WAIS-III: Wechsler Adult Intelligence Scale, Third edition; 15-OT: The 15-Objects test; SKT: Syndrom Kurtz Test; CDR: The Clinical Dementia Rating; NPI-Q: Neuropsychiatric Inventory-questionnaire; CAMCOG: The cognitive and self-contained part of the Cambridge Examination for Mental Disorders of the Elderly; VAT: Visual Association Test; RAVLT: Rey Auditory Verbal Learning Test; COWAT: Controlled Oral Word Association Test; VOSP: The Visual Object and Space Perception Battery; TMT: Trail Making Test; FAB: Frontal Assessment Battery; DAD: the Disability Assessment for Dementia; Amsterdam IADL-Q: the Amsterdam Instrumental Activities of Daily Living questionnaire.

Supplementary table 2

A. Markers succesfully genotyped

GENE	SNP_ID	Sequenom Plex	Authors
ABCA7	rs3752246	plex1	Naj et al.
ABCA7	rs3764650	plex1	Hollingworth et al.
ADAMST20	rs7295246	Plex3	Lambert et al.
APOE (e2)	rs7412	plex1	Strittmatter et al.
APOE (e4)	rs429358	plex1	Strittmatter et al.
BIN1	rs744373	plex1	Seshadri et al.
BIN1	rs7561528	plex1	Wijsman et al.
CASS4	rs7274581	Plex3	Lambert et al.
CD2AP	rs1094836	plex1	Lambert et al.
CD33	rs3865444	plex1	Naj et al.
CLU	rs1113600	plex1	Harold et al.
CLU	rs9331888	plex1	Lambert et al.
CR1	rs3818361	plex1	Lambert et al.
CR1	rs6656401	plex1	Lambert et al.
ECHDC3	rs7920721	Plex3	Lambert et al.
EPHA1	rs1080802	plex2	Naj et al.
FERMT2	rs1712594	Plex3	Lambert et al.
FRMD4A	rs1731422	plex1	Lambert et al.
FRMD4A	rs7081208	plex1	Lambert et al.
GAB2	rs2373115	plex1	Reiman et al.
HS3ST1	rs6448799	Plex3	Lambert et al.
INPP5D	rs3534966	Plex3	Lambert et al.
MEF2C	rs190982	Plex3	Lambert et al.
MS4A4A	rs4938933	plex1	Naj et al.
MTHFD1L	rs1175466	plex2	Naj et al.
NDUFAF6	rs7818382	Plex3	Lambert et al.
NME8	rs2718058	Plex3	Lambert et al.
None	rs6678275	Plex3	Lambert et al.
PICALM	rs3851179	plex1	Harold et al.
PICALM	rs561655	plex1	Naj et al.
PILRA	rs2405442	Plex3	Lambert et al.
PILRA	rs3499583	Plex3	Lambert et al.
PTK2B	rs2883497	Plex3	Lambert et al.
SCIMP	rs7225151	Plex3	Lambert et al.
SLC24A4/R	rs1049863	Plex3	Lambert et al.
SORL1	rs1121834	plex2	Mayashita et al.
SORL1	rs2070045	plex1	Li et al.
SORL1	rs641120	plex1	Liu et al.
SPPL2A	rs8035452	Plex3	Lambert et al.
TOMM40	rs2075650	plex1	Harold et al.
TREML2	rs9381040	Plex3	Lambert et al.
ZCWPW1	rs1476679	Plex3	Lambert et al.

B. Markers selected but discarded during the genotyping project

GENE	SNP	Note	Datasets with QC issues
AP2A2	rs1075166	HWE violations or high missingness	AGECODE, DCN,ACE,ADC
CD2A2AP	rs9349407	Rejeted by sequenom	not applicable
CELF1	rs1083872	HWE violations	AGECODE, DCN,ACE
DSG2	rs8093731	genotyping faliure. Low MAF	AGECODE, DCN,
EPHA1	rs1176755	genotyping faliure	AGECODE, DCN,ACE,ADC
HLA-DrB5	rs9271192	Rejeted by sequenom	not applicable
MS4A6A	rs610932	Rejeted by sequenom	not applicable
TRIP4	rs7461516	HWE violations	ACE
ZCWPW1	rs3491992	genotyping faliure	AGECODE, DCN,ACE,ADC

Reference	PGS scores
Nat Genet. 2011 May;43(5):436-41	no
Nat Genet. 2011 May;43(5):429-35	PGS1
Nat Genet. 2013 Dec;45(12):1452-8	no
Proc Natl Acad Sci U S A. 1993 Mar 1;90(5):1977-81.	no
Proc Natl Acad Sci U S A. 1993 Mar 1;90(5):1977-81.	no
JAMA. 2010 May 12;303(18):1832-40	PGS1
PLoS Genet. 2011 Feb;7(2):e1001308	no
Nat Genet. 2013 Dec;45(12):1452-8	PGS2
Nat Genet. 2013 Dec;45(12):1452-8	PGS1
Nat Genet. 2011 May;43(5):436-41	PGS1
Nat Genet. 2009 Oct;41(10):1088-93	PGS1
Nat Genet. 2009 Oct;41(10):1094-9	no
Nat Genet. 2009 Oct;41(10):1094-9	no
Nat Genet. 2009 Oct;41(10):1094-9	PGS1
Nat Genet. 2013 Dec;45(12):1452-8	no
Nat Genet. 2011 May;43(5):436-41	PGS1
Nat Genet. 2013 Dec;45(12):1452-8	PGS2
Mol Psychiatry. 2013 Apr;18(4):461-70	no
Mol Psychiatry. 2013 Apr;18(4):461-70	no
Neuron. 2007 Jun 7;54(5):713-20.	no
Nat Genet. 2013 Dec;45(12):1452-8	no
Nat Genet. 2013 Dec;45(12):1452-8	PGS2
Nat Genet. 2013 Dec;45(12):1452-8	PGS2
Nat Genet. 2011 May;43(5):436-41	PGS1
PLoS Genet. 2010 Sep 23;6(9):e1001130	no
Nat Genet. 2013 Dec;45(12):1452-8	no
Nat Genet. 2013 Dec;45(12):1452-8	PGS2
Nat Genet. 2013 Dec;45(12):1452-8	no
Nat Genet. 2009 Oct;41(10):1088-93	PGS1
Nat Genet. 2011 May;43(5):436-41	no
Nat Genet. 2013 Dec;45(12):1452-8	no
Nat Genet. 2013 Dec;45(12):1452-8	no
Nat Genet. 2013 Dec;45(12):1452-8	PGS2
Nat Genet. 2013 Dec;45(12):1452-8	no
Nat Genet. 2013 Dec;45(12):1452-8	PGS2
PLoS One. 2013;8(4):e58618.	PGS2
Neurobiol Dis. 2008 Feb;29(2):293-6	no
J Alzheimers Dis. 2009;18(1):51-64	no
Nat Genet. 2013 Dec;45(12):1452-8	no
Nat Genet. 2009 Oct;41(10):1088-93	no
Nat Genet. 2013 Dec;45(12):1452-8	no
Nat Genet. 2013 Dec;45(12):1452-8	PGS2

Author	Reference
Lambert et al.	Nat Genet. 2013 Dec;45(12):1452-8
Naj et al.	Nat Genet. 2011 May;43(5):436-41
Lambert et al.	Nat Genet. 2013 Dec;45(12):1452-8
Lambert et al.	Nat Genet. 2013 Dec;45(12):1452-8
Naj et al.	Nat Genet. 2011 May;43(5):436-41
Lambert et al.	Nat Genet. 2013 Dec;45(12):1452-8
Harold et al.	Nat Genet. 2009 Oct;41(10):1088-93
Ruiz et al.	Transl Psychiatry. 2014 Feb 4;4:e358
Lambert et al.	Nat Genet. 2013 Dec;45(12):1452-8

Supplementary Online Content

Genome-wide significant risk factors for Alzheimer's disease: Role in progression to dementia due to AD among subjects with mild cognitive impairment.

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Description of datasets.

a. **German Series (AgeCoDe and DCN cohorts) MCI dataset**

AgeCoDe

Between 2003 and 2004, a total of 3327 participants (aged >74 years) were recruited via 138 general practitioners for the AgeCoDe cohort across six study centers in Germany.[1,2] Recruitment, inclusion and exclusion criteria, and assignment of a MCI diagnosis in the AgeCoDe study are described in detail eslewhere.[1] For the purposes of the present study, n=853 MCI cases were selected. The inclusion criteria were: (i) assignment of a diagnosis of MCI at study baseline or at 18 month interval follow up assessments thereafter; and (ii) a minimum of one follow-up assessment after MCI diagnosis with information on progression / non-progression to incident AD dementia. In cases where personal interview was not possible, the Global Deterioration Scale (GDS)[3] and the Blessed Dementia Rating Scale (BDRS)[4] were completed by the interviewer using information provided by an informant and the general practitioner.

Neuropsychological Assessment

In the personal interview, all AgeCoDe participants were assessed using the Structured Interview for diagnosis of Dementia of Alzheimer type, Multi-infarct Dementia, and Dementia of other etiology according to Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV)[5] and the International Classification of Diseases (ICD)-10[6] (SIDAM).[7,8] The SIDAM[7,8] contains a 55-item neuropsychological test battery;[9,] a 14-item scale for the assessment of activities of daily living (ADL);[1] and the Hachinski Rosen-Scale.[10] The 55-item neuropsychological battery includes all 30 items of the Mini Mental State Examination (MMSE)[11] and evaluates the following cognitive domains: orientation, registration, attention and calculation, recall, language, and visuoconstructional abilities.

Diagnosis of MCI and Incident AD Dementia

All diagnoses were assigned by consensus at a multidisciplinary case conference in accordance with the criteria of the International Working Group on Mild Cognitive Impairment.[12] Assignment of a MCI diagnosis required the absence of dementia according to the ICD-10[6] evidence of cognitive decline according to self- or informant report; impairment in the performance of objective cognitive tasks and/or evidence of decline over time in the performance of objective cognitive tasks;[1] maintained baseline ADL or minimal impairment only in complex instrumental functions;[2] and a Clinical Dementia Rating (CDR) of 0.5.[13]

Incident dementia was diagnosed according to the DSM-IV criteria,[5] which are included as a diagnostic algorithm in the SIDAM.[7,8] Assignment of a diagnosis of AD in incident dementia cases was based on: (i) the criteria for probable AD of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA)[14,15]; (ii) a GDS score of ≥ 4 [3]; and (iii) a CDR of 1.[13] Mixed dementia (MD) was defined as AD with cerebrovascular disease. This diagnosis was assigned in cases with a history of cerebrovascular events with no temporal relationship to cognitive decline. For the purposes of the present analyses, MD and AD cases were combined (n=209). MCI cases were defined as stable (n=295) when the respective subjects did not convert to AD or MD, or remained stable at the end of the follow-up period.

Dementia Competence Network (DCN) dataset

The DCN cohort included n= 812 MCI patients with a minimum age of 50 years who were recruited at 14 University Hospital memory clinics across Germany between 2003 and 2005.[9] Recruitment, inclusion and exclusion criteria, and assignment of a MCI diagnosis in the DCN study are described in detail elsewhere.[9] Briefly, MCI patients were recruited from among individuals who had sought evaluation at the participating memory clinics, and in whom organic cognitive impairment was suspected.

Neuropsychological Assessment

All subjects in the DCN cohort were evaluated using standardized neuropsychological tests sensitive to verbal learning and memory, visual learning and memory, word fluency, naming, visuoconstruction, cognitive speed, and executive function.[9]

Diagnosis of MCI and Incident AD Dementia

Clinical diagnoses of MCI were assigned at multidisciplinary case conferences at the local study centers, and were confirmed through general physical-, neurological-, and psychiatric examinations performed according to the 2004 criteria of Winblad et al.[9,12] Standard laboratory investigations and structural magnetic resonance imaging scans of the brain were performed. The DCN was designed to include a broad range of patients at risk of developing dementia. Therefore, instead of the classical MCI definition, which requires a confirmed deficit in memory in the presence of otherwise normal cognitive abilities, a broader definition of MCI was used. In applying this broader diagnosis, the core features considered were reported cognitive deficits in daily living and an objective decline in cognitive abilities (more than 1 SD) in at least 1 of the following domains, as elicited by standardized neuropsychological tests: verbal learning and memory, nonverbal learning and memory, word fluency, naming, visuoconstruction, cognitive speed, and executive purpose. Minor changes in complex ADL were tolerated, e.g. dealing with financial affairs and orientation difficulties in unfamiliar surroundings (total Bayer-ADL (B-ADL) score < 4)[9] In addition, all patients were required to have a CDR of 0.5.[13]A maximum of three annual follow-up study assessments were performed .

A diagnosis of incident dementia was assigned on the basis of ICD-10 criteria.[6] A diagnosis of AD was assigned according to the NINCDS-ADRDA criteria for probable AD.[14,15] All AD subjects had a CDR[13] of 1 (n=76). Subjects who did not convert to AD, or who remained stable during follow-up, were classified as having stable MCI (n=521).

Ethical Issues

The present study was approved by the respective ethics committees, and written informed consent was obtained from all participants prior to inclusion. All study procedures complied with national legislation and the Code of Ethical Principles for Medical Research Involving Human Subjects of the World Medical Association. Detailed descriptions of the AgeCoDe and DCN studies are available elsewhere.[1,2,9]

b. **Fundació ACE in Barcelona (ACE) MCI dataset**

The ACE MCI cohort comprises 1245 individuals aged > 48 years who were recruited and assessed at the Diagnostic Unit of Fundació ACE (Barcelona, Spain) between January 2006 and July 2013. All diagnoses were assigned by consensus at a case conference attended by neurologists, neuropsychologists, and social workers. Recruitment, inclusion and exclusion criteria, and assignment of a diagnosis of MCI in the ACE dataset are described in detail elsewhere.[16] All subjects underwent a minimum of one follow-up assessment.

Neuropsychological Assessment

All MCI patients completed the neuropsychological battery of Fundació ACE (NBACE).[17,18] This diagnostic procedure included tests sensitive to processing speed, orientation, attention, verbal learning and memory, language, spatial, perceptual spatial perception, praxis, and executive functions.

Diagnosis of MCI and Incident AD Dementia

A diagnosis of MCI was assigned according to the Petersen criteria[19,20] and the classification of Lopez et al.[21,22,16] All MCI patients had measurable cognitive impairments that were insufficient to warrant a diagnosis of dementia. Minor impairments in complex ADL were allowed. None of the patients reported deficits in general intellectual abilities, and all patients were autonomous at the time of enrolment. All subjects had a CDR of 0.5,[13] and were assessed using the MMSE;[11] the Hachinski Ischemia Scale;[10] the BDRS;[4] and the Neuropsychiatric Inventory Questionnaire (NPI-Q).[23] Subjects who converted to AD[14,15] during the course of the study (n=395) were defined as MCI converters. For the purposes of the present analyses, AD and MD

were combined. All AD and MD subjects had a CDR of 1.[13] Subjects who did not convert to AD (n=776) or MD (n=548) were defined as MCI non-converters.

Ethical Issues

The study was approved by the respective ethics committees, and written informed consent was obtained from all participants prior to inclusion. The study protocol complied with national legislation and the Code of Ethical Principles for Medical Research Involving Human Subjects of the World Medical Association.

c. Amsterdam Dementia Cohort (ADC) MCI Dataset

The ADC cohort included 316 MCI patients aged > 42 years who had attended the memory clinic of the Alzheimer Center at the VU University Medical Center (VUmc) between 2000 and 2013. All diagnoses were assigned by consensus at a case conference attended by neurologists, neuropsychologists, and nurses. Recruitment, inclusion and exclusion criteria, and assignment of a diagnosis of MCI in the ADC dataset are described in detail elsewhere.[24] Following the exclusion of samples that failed quality control, a total of n=306 cases with data from at least one annual follow-up assessment were available for the present analyses.

Neuropsychological Assessment

All subjects underwent a neuropsychological assessment, which included tests sensitive to global cognitive decline, memory, language, visuospatial functioning, attention, and executive functions.[24]

Diagnosis of MCI and Incident AD Dementia

Prior to the beginning of 2012, the Petersen criteria were used to assign a diagnosis of MCI[19,20] . Thereafter, the National Institute on Aging-Alzheimer's Association (NIA-AA) criteria for MCI were applied.[25] Inclusion was restricted to MCI patients for whom cerebrospinal fluid (CSF) was available.[24] All diagnoses were assigned at a multidisciplinary consensus conference without reference to the results of the CSF investigation. All subjects were assessed using the MMSE;[10] Neuropsychiatric Inventory (NPI);[26] the abbreviated Geriatric Depression Scale (GDS);[27] the Disability Assessment for Dementia (DAD);[28] the Amsterdam Instrumental Activities of Daily Living questionnaire (Amsterdam IADL Questionnaire);[29,30] and all had a CDR of 0.5.[13]All subjects who converted to dementia had a CDR of 1.[13] Subjects who converted to AD[14,15] (n=113) during the study period were defined as MCI converters. Subjects who did not convert to AD (n=81), or who remained stable during follow-up (n=112), were defined as MCI non-converters.

Ethical Issues

The medical ethics committee of the VU University Medical Center approved the study protocol for the use of clinical data for research purposes and the biobank protocol for storage and use of DNA. Written informed consent was obtained from all individuals prior to inclusion.

Genotyping Procedures

DNA Extraction

DNA was extracted using standard procedures. This was performed for a total of 3226 MCI samples from the three datasets: the German Series (DCN, n=812 and AgeCoDe, n=853); Fundació ACE (ACE, n=1245); and the MCI dataset of the Amsterdam Dementia Cohort (ADC, n=316).

Single Nucleotide Polymorphism (SNP) Selection Criteria

A total of 51 SNPs with reported evidence of association with AD risk were selected for replication and polygenic score construction (supplementary Table 2). Nine of these SNPs were discarded due to technical problems. Thus 42 SNPs located in 31 different AD associated loci were investigated in the present study. Nine of these SNPs were identified in the recent International Genomics of Alzheimer's Project (IGAP)

consortium study.[31] Other SNPs that had been identified by IGAP but which did not reach genome wide significance were chosen for univariate analysis but not for polygenic score analysis. The SNP rs2075650, which is located at *TOMM40*, was included in the analysis as a proxy of APOE effect. The SNPs rs8412 and rs429358, which determine the *APOE*-ε2/-ε3/-ε4 alleles,[32] were encoded as individual SNPs, and *APOE*-ε2/-ε3/-ε4 diplotype conventional nomenclature.

Genotyping

The primer molecules for the multiplex reaction were selected using the Assay Design Suite tool (www.mysequenom.com, Sequenom, San Diego, California, USA). Of the 51 SNPs, three SNPs (rs9349407, rs9271192, rs610932, supplementary table 2B) were excluded due to problems during the design phase. The primer sequences and assay conditions for the genotyped SNPs are available upon request. In total, 48 SNPs were genotyped in all four series, representing 46 non-APOE-related SNPs and two SNPs that define the APOE-ε2/-ε3/-ε4 haplotypes, i.e., rs7412 and rs429358 (supplementary table 2B). *APOE*-ε2/-ε3/-ε4 haplotypes were available for the majority of individuals. The SNP rs429358, whose alleles correspond to ε4 and non-ε4 carriers, was also extracted from the *APOE* haplotypes.

Quality Control

Only SNPs with a call rate of ≥ 95% and a Hardy-Weinberg Equilibrium (HWE) p-value of >0.001 in all datasets were used in the subsequent analyses (Table 1). The SNPs major and minor alleles, and allelic frequencies, were consistent with previous reports. The overall genotype conversion rate during the genotyping process was 96.7%.

Of the 1101 individuals from the German datasets (DCN and AgeCoDe cohorts), 285 individuals converted to AD or MD during follow up. Subjects who converted to non-AD forms of dementia were censored at that time-point. Forty-six AD patients were excluded due to a lack of follow-up data, and one individual was excluded due to the absence of a diagnosis of either MCI or AD. Eight MCI patients had been recruited twice and were therefore removed once. Two AD and seven MCI patients were excluded due to missing genotypes.

Of the 48 genotyped SNPs, six were excluded due to genotyping failure (rs11767557 and rs34919929), HWE violations (rs10751667, rs10838725, and rs74615166), or a very low minor allele frequency (MAF) (rs8093731; MAF <0.01). Since the German sample was collected from two different sources, systematic differences in the demographics (gender and age) were tested. For gender, no significant correlation was found with either the F-test (p=0.97), or a t-test (p=0.81). In contrast, both the F-test ($p=2.2 \times 10^{-16}$) and the Welch test ($p=2.2 \times 10^{-16}$) revealed systematic deviation for age. This was attributed to differences in recruitment methodology and demographics, since the AgeCoDe cohort comprised patients with a very late onset disorder. These systematic differences were considered in all subsequent analyses. Accordingly, after quality control analyses and internal discussion, the two German series were analyzed separately.

The ACE dataset comprised 1245 MCI patients, of whom 443 converted to AD or MD. No duplicated samples were detected. Six SNPs were removed due to genotyping failure (rs11767557, rs34919929); HWE violation (rs10838725, rs74615166); high missing rate (rs10751667); or very low MAF (rs8093731).

The ADC dataset comprised 316 MCI patients, of whom 113 converted to AD. No duplicated samples were detected. A total of 10 subjects were removed due to low genotype quality. Three SNPs were removed due to either genotyping failure (rs11767557, rs34919929), or high missing rate (rs10751667). No major HWE violations were detected in the ADC series.

Overall, 42 genotyped markers fulfilled the quality control criteria (supplementary table 2A). Two of these correspond to SNPs used to genotype APOE (rs7412 and rs429358). Main table 2 therefore displays univariate results for the remaining markers (40 SNP markers genotyped in all datasets).

Statistical Analysis

Methods from survival analysis were used to investigate the influence of genetic markers and demographic factors on the rate of progression from MCI to AD dementia.

a. Univariate analysis using Cox proportional hazards models

Proportional hazards models relate the time taken to the occurrence of a given event to a set of covariates that may be related to the elapsed time-period. The applied hazards model is given by

$$h(t,z) = h_0(t)\, e^{\beta_{snp} z_{snp} + \beta_{age} z_{age} + \beta_{sex} z_{sex} + \beta_{\varepsilon 4} z_{\varepsilon 4} + \beta_{edu} z_{edu}} . \qquad (1)$$

The hazard rate function h(t,z) is calculated according to accumulated information concerning the individuals who have converted during the observation period, the minimum period of time to AD dementia, and the entire period of observation. If an individual has not converted during the observation period, this period is called "censor time". Similarly, individuals who have not converted during that time-period are classified as "censored". The unknown function $h_0(t) = \lim_{\Delta t \to 0} P(t \le \tau \le t + \Delta t | t \le \tau)/\Delta t$ describes the hazard rate in the absence of covariates. The regression coefficients β_i of a proportional hazards model can be calculated from the logarithm of the hazard ratio, i.e. the ratio between hazard rate functions whose corresponding covariate states differ by one. This hazard ratio, $\exp(\beta_i)$, can be interpreted as a per-unit relative risk increase of the parameter. The unique effect of an increase of a parameter by one unit is multiplicative with respect to the hazard rate.

In the unadjusted hazards model, The parameters were evaluated in the following order: (i) the number of minor alleles $z_{snp} \in \{0,1,2\}$ in the individual for the investigated SNP (model 0, unadjusted, or crude model); (ii) the age at study recruitment z_{age} (continuous); (iii) gender $z_{sex} \in \{1,2\} \widehat{=} \{\text{male,female}\}$ (model 1); (iv) *APOE*-ε4 status, as defined by $z_{\varepsilon 4} \in \{0,1\} \widehat{=} \{\text{non-carrier,carrier}\}$; and (v) education as additional covariates (model 2). The analysis was performed using the R (The R project for scientific computing, http://www.r-project.org/) package "survival", where the ties were handled using the Efron method.[33] The response (the left hand side of the equation) was calculated in a non-parametric manner using R's function *Surv()*. P-values for the covariates were obtained using the Wald test by testing the full model against the model without the covariate of interest

b. Polygenic scores

Polygenic analysis is performed to test a polygenetic model, in which multiple common SNPs, which show no apparent association with the trait individually, show a collective effect on the phenotype in aggregate. Polygenic scores were calculated according to Purcell et al.,[34] with the inclusion of a normalization factor.

Polygenic Score Construction

Polygenic scores were constructed using sets of AD associated loci identified in recent genome wide association studies (GWAS). For polygenic score construction, we selected only a single SNP per locus. Proxy SNPs are not independent, and therefore including both SNPs in polygenic score (PGS) may provide null information due to Linkage disequilibrium (LD) between SNPs. The inclusion of SNPs in the polygenic score was based on definitive evidence of association in large meta-GWAS reported by four large AD genetics consortia (EADI, ADGC, GERAD, and CHARGE). Since the well-known association of *APOE*-ε4 with AD is present in our study cohorts, the *APOE*-region was excluded from our calculation of PGS. Consequently, polygenic score 1 (PGS1) represented the nine (see supplementary Table 3. Part A) well-known AD-associated SNPs reported prior to publication of the IGAP consortium results (see upper part of Table 2 in Lambert et al.).[31] Polygenic score 2 (PGS2) comprised nine (see supplementary Table 3. Part B) of the 11 novel AD-associated SNPs identified by IGAP (remainder of Table 2 in Lambert et al.).[31] Polygenic score 3 (PGS3) comprised the full list of 18 associated SNPs.

The reasons for designing three different PGS are four-fold. First, the markers of PGS1 are the best AD-associated SNP set reported to date. Second, First, all SNPs of PGS1 have larger effect sizes than IGAP identified signals, since they were identified using less than half of the sample used in the IGAP effort. Third, with the exception of CD33, these SNPs have been re-confirmed in the large replication dataset included in

the IGAP effort[31], as well as in many other independent studies. Fourth, the PGS2 SNPs, which were discovered by IGAP, still await a large independent replication effort.

The polygenic score z_{pgs} for a given individual was calculated as the sum of manifest risk alleles of all considered SNPs, with each one being weighted with the logarithm of its odds ratio.

$$z_{pgs} = \frac{\sum_{i\epsilon\{snps\}} z_i \ln OR_i}{\sum_{i\epsilon\{snps\}} \ln OR_i}, \qquad (2)$$

Here, a risk allele was characterized as one that increases the odds of AD susceptibility in a case-control analysis. For this purpose, allele information and odds ratios were gathered from Lambert et al.[31] To ensure that interpretation of the resulting hazard ratio remained possible, the score was divided by the sum of the odds ratios' logarithms, such that the resulting score was a number between zero and two. An advantage of this approach is that it creates comparable PGSs, since otherwise hazard ratios would strongly depend on the weights. As suggested by previous authors,[34] missing genotypes were imputed with their expected values, which are calculated as two times the risk allele frequency. The latter was taken from the CEU population.[35] Individuals with a significant number (>1/3) of missing contributing SNPs were excluded from subsequent analysis.

Polygenic Score Evaluation

For each individual, the aforementioned polygenic scores were calculated. The polygenic score was used as a dose, and the proportional hazards model was employed in correspondence to the model applied for analyzing single SNPs

$$h(t,z) = h_0(t)\, e^{\beta_{pgs} z_{pgs} + \beta_{miss} z_{miss} + \beta_{age} z_{age} + \beta_{sex} z_{sex} + \beta_{\epsilon 4} z_{\epsilon 4} + \beta_{cht} z_{edu}}, \qquad (3)$$

where $z_{pgs} \in [0,2]$ as mentioned above. z_{miss} (continuous) is given by the sum of the odds ratios' logarithms from the missing genotypes only ($z_{miss} = \sum_{i\epsilon\{snps|missing\}} \ln OR_i$). This covariate symbolizes a cross check for correlation between the hazard and the missingness, and is not substantial. The other covariates are explained in section 3.a.

a. Interaction Analysis and Plots

The effect of rs11136000, polygenic score 1, and polygenic score 2 were also analyzed after stratification for the presence of the *APOE*-ε4 allele. Rs11136000 *CLU* and polygenic score 1, which exhibited significant effects during meta-analysis, were further explored by introducing interaction terms in Cox proportional hazard models with all co-variables (*APOE*-ε4 status, education, gender, and age). Stratified analyses, interaction calculations, and graphic representations were conducted using the IBM Statistical Package for the Social Sciences (SPSS) software v19.0.0.

b. Meta-Analysis

Meta-analysis techniques were used to estimate global effects of SNPs and the polygenic score. These studies were conducted using the standard fixed effect approach of the Yet Another Meta-Analysis Software (YAMAS) software v.931.68.[36]

c. Effect of age and gender on MCI to AD dementia progression

The demographic characteristics of the four cohorts are summarized in Table 1. Final effective sample size for the meta-analysis was 2578 subjects for models 0 (without covariates) and 1 (age and gender adjusted), and 2393 for model 2 (age, gender APOE, and education adjusted). In the AgeCoDe and ACE series, the average age at MCI diagnosis was significantly higher compared to the DCN and ADC series.

In all four cohorts, age significantly increased the annual chance of MCI to AD dementia progression (ACE $p=1.00 \times 10^{-11}$; AgeCoDe $p=2.02 \times 10^{-6}$; DCN $p=6.06 \times 10^{-6}$; and ADC $p=1.0 \times 10^{-3}$). The p-value differences reflected differences in the statistical power of each dataset rather than between series heterogeneity ($I^2=4.9$). Meta-analysis of age revealed a statistically significant effect in MCI to AD dementia progression. The estimated average annual increase in MCI to AD dementia progression risk was 5.8% (Hazard risk (HR)=1.058[1.05-1.07], p=2.16 x 10-21).

Compared to age, the effect of gender on MCI to AD dementia progression was less clear. Statistically significant effects were observed in the DCN, ACE, and ADC series, with the HR ranging from 1.38 ($p=4.0 \times 10^{-3}$, ACE) to 1.71 (p=0.02, DCN). However, no significant effects were found for AgeCoDe (HR=0.83, p=0.243). For gender, heterogeneous effects were found across the four study cohorts ($I^2=72.5$) and the meta-analysis identified a non-significant moderately higher risk for females (HR=1.292, p=0.096, model 1).

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Supplementary table 3: Polygenic Scores (PGS) construction

SNP	Chromosome	Position	Closest gene	Alleles			Odds Ratio	Risk-allele frequency
				minor	major	risk		
Part A, Contributing Markers to PGS1								
rs6656401	1	207692049	*CR1*	A	G	A	1.18	0.197
rs744373	2	127894615	*BIN1*	C	T	C	1.22	0.409
rs10948363	6	47487762	*CD2AP*	G	A	G	1.10	0.292
rs10808026	7	143099133	*EPHA1*	A	C	C	1.11	0.842
rs11136000	8	27464519	*CLU*	T	C	C	1.16	0.621
rs4938933	11	60034429	*MS4A4A*	C	T	T	1.11	0.533
rs3851179	11	85868640	*PICALM*	A	G	G	1.15	0.642
rs3764650	19	1046520	*ABCA7*	G	T	G	1.15	0.190
rs3865444	19	51727962	*CD33*	T	G	G	1.06	0.693
Part B, Contributing Markers to PGS2								
rs35349669	2	234068476	*INPP5D*	T	C	T	1.08	0.488
rs190982	5	88223420	*MEF2C*	G	A	A	1.08	0.592
rs1476679	7	100004446	*ZCWPW1*	C	T	T	1.10	0.713
rs2718058	7	37841534	*NME8*	G	A	A	1.08	0.627
rs28834970	8	27195121	*PTK2B*	C	T	C	1.10	0.366
rs11218343	11	121435587	*SORL1*	C	T	T	1.30	0.961
rs10498633	14	92926952	*SLC24A4/RIN3*	T	G	G	1.10	0.783
rs17125944	14	53400629	*FERMT2*	C	T	C	1.14	0.092
rs7274581	20	55018260	*CASS4*	C	T	T	1.14	0.917

Artículo IV

6.4. Exploring genetic effects of Alzheimer's disease loci in mild cognitive impairment neuropsychological endophenotypes.

Espinosa A, Hernández-Olasagarre B, Moreno-Grau S, Hernández I, Rosende-Roca M, Mauleón A, Vargas L, Lafuente A, Rodríguez-Gómez O, Abdelnour C, Sánchez D, Gil S, Santos M, Sanabria A, Ortega G, Sotolongo-Grau O, Pérez A, Ibarria M, Ruiz S, Montrreal L, Cañabate P , Moreno M, Preckler S, Aguilera N, de Rojas I, Orellana A, Valero S, Alegret M, Tárraga L, Boada M, Ramírez A, Ruiz A.

En revisión.

Exploring genetic effects of Alzheimer's disease loci in Mild Cognitive Impairment neuropsychological endophenotypes

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Abstract

The role of genetic risk markers of Alzheimer's disease (AD) on neuropsychological endophenotypes among subjects with Mild Cognitive Impairment (MCI) has been scarcely studied. We therefore investigated the role of well-known AD-associated single nucleotide polymorphism (SNP) in comprehensive neuropsychological endophenotypes routinely evaluated during diagnose of MCI, AD and other dementias. For the analysis, the MCI dataset (n=1245) from Fundació ACE (ACE) was included. Associations between neuropsychological endophenotypes and genetic markers were measured through a multivariate linear regression analysis using PLINK and graphical representation of data were performed using Heat Maps. Analysis were performed in both the total MCI sample and also stratified into Probable amnestic (Pr-aMCI) (*n* = 262), probable non-amnestic (Pr-naMCI) (*n* = 76), possible amnestic (Pss-aMCI) (*n* =549), and possible non-amnestic (Pss-naMCI) (*n* = 358), single or multiple domain. In the total sample MCI group, a significant association was only observed between *APOE*-ε4 and Learning, Delayed recall and Recognition of the WMS-III endophenotypes. Additionally, *APOE*-ε4 showed also association with Learning (β= -1.37, p=5.82x10^{-5}) in the Pss-aMCI phenotype. In Pr-aMCI, the *HS3ST1* locus (rs6448799) showed association with the Backward Digits endophenotype (β=0.52, p=7.57x10^{-5}). Finally, the *AP2A2* locus (rs10751667) was found associated with Repetition in Pr-aMCI (β= -0.19, p=5.34x10^{-6}). Our findings provide evidences supporting the specific effect of well-known AD-associated on single cognitive domains. However, further studies in larger longitudinal MCI samples are now warranted to replicate or not our data, and to disentangle the genetic underpinning cognitive function in MCI and AD.

Keywords: Alzheimer's disease, Mild Cognitive Impairment, Neuropsychological endophenotypes, GWAS, SNP, DNA.

Introduction

Alzheimer's disease (AD) is the most common cause of dementia (Blennow et al., 2006), representing 50–60% of all cases. The risk of AD results from the complex interaction of genetics, epigenetics, and environmental factors. In AD, the genetic factors contributing to the disease have been estimated in up to 79% (Wingo et al., 2012). Research into the genetics of AD has been tremendously successful in identifying risks factors for AD ever since the developments of high throughput genomics and genome-wide association studies. Thus in 2013, the International Genomics of Alzheimer's Project (IGAP) consortium analyzed >74,000 individuals identifying 21 risk loci for AD besides *APOE*, with 11 representing novel susceptibility factors (Lambert et al. 2013). However, while these factors unraveled factors affecting the general susceptibility to AD, their contribution to specific endophenotypes, such as disease progression or cognitive functions, is less clear. Thus, for example, research on disease progression to AD dementia has shown consistent association only with *APOE* (Elias-Sonnenschein et al., 2011; Lacour et al., 2016). Endophenotypes are quantitative traits (QTs) that are supposed to be closer to the related pathophysiology behind. Hence by using quantitative endophenotypes instead of qualitative case/control status as the phenotype for a genetic study, research has been able to reduce heterogeneity in clinical diagnosis, thus increasing power to detect genetic associations. In addition, this approach can provide more specific hypotheses for the biological pathways

by which associated variants modulate disease progression. As a proof of principle of this strategy in AD, we and others have identified and replicated genetic factors by combining GWAS data with biomarkers of AD, such as cerebrospinal fluid levels of amyloid-42, Tau and phosphorylated Tau. Crucially, the samples size included in these studies are several orders of magnitude smaller than those used in a case/control setup.

Along this lines, neuropsychological tests represent a good source of endophenotypes for AD, especially those tests related to episodic memory impairments (MacAulay et al., 2015; Ramanan et al., 2015; Ramanan and Saykin, 2015). Impairment of episodic memory is usually the earliest clinical symptom in AD dementia and in the prodromal phase of AD dementia called Mild Cognitive Impairment (MCI) (Estévez-González et al., 2003; Grundman et al., 2004; Morris et al., 2001; Moulin et al., 2004; Petersen et al., 1999)(Dubois et al., 2010). A recent study (Espinosa et al., 2013) has shown that those Probable amnestic (Pr-aMCI) that is, with the absence of comorbidities that could explain cognitive deficits (i.e., cerebrovascular disease, anxiety or depression) and memory storage impairment (i.e., impaired recall and recognition) had 8.5 times more risk of conversion to dementia, mainly Alzheimer's disease (AD), than those with the possible non-amnestic (Pss-naMCI) condition. Interestingly, it is anticipated that the genetic factors involved in AD susceptibility will influence cognitive endophenotypes in MCI stages. Hence, research into the cognitive endophenotype in MCI may represent an interesting approach to identify individuals with incipient AD or at risk of developing AD. Although altered cognitive endophenotypes (e.g. episodic memory impairments) might represent a reliable early manifestation of disease, the systematic investigation of the relationships between AD genetic risk factors and their endophenotypic expressions is still in its infancy.

The aim of the present study was therefore to explore the involvement of AD related genes in comprehensive neuropsychological endophenotypes routinely evaluated during diagnose MCI, AD, and other dementias. Using genetic association techniques and by combining different baseline clinical MCI phenotypes from Fundació ACE (ACE) MCI dataset (n= 1245 subjects), we explored the association between the AD loci with neuropsychological metrics obtained in MCI subjects.

Methods

Patients

The present cohort comprised MCI patients from the Fundació ACE in Barcelona (ACE, n = 1245)(Espinosa et al., 2013; Lacour et al., 2016) aged > 60 years who were recruited and assessed at the Diagnostic Unit of Fundació ACE (Barcelona, Spain) between January 2006 and July 2013. Demographics, clinical characteristics and *APOE* differences between MCI phenotypic groups are shown in Table 1. All MCI diagnoses were assigned by consensus at a case conference attended by neurologists, neuropsychologists, and social workers. Recruitment, inclusion and exclusion criteria, and assignment of a diagnosis of MCI in the ACE dataset are described in detail elsewhere (Espinosa et al., 2013). Briefly, MCI subjects were classified into Pr-aMCI, probable non-amnestic (Pr-naMCI), possible amnestic (Pss-aMCI) (*n* =549) and Pss-naMCI, single or multiple domain fulfilling Petersen's criteria (Ronald C Petersen, 2004; Petersen et al., 1999) and tacking into account Lopez et al. (Lopez et al., 2007, 2003) classification, but extending it to the non-amnestic MCI groups (single or multiple domain). All MCI subjects were classified as possible MCI when there

were comorbidities (i.e., cerebrovascular disease, anxiety or depression) that could explain or contribute to cognitive deficits; and they were classified as probable MCI when there were none (Espinosa et al., 2013). All subjects underwent a minimum of one follow-up assessment (average follow-up: 29.4 months; range: 6-68 months). Pheno-conversion criteria has been published elsewhere (Lacour et al., 2016).

Neuropsychological endophenotypes

All MCI patients completed the neuropsychological battery of Fundació ACE (NBACE) (Alegret et al., 2013, 2012). This diagnostic procedure included the assessment of eight cognitive functions comprising tests sensitive to processing speed, orientation, attention, verbal learning and memory, language, visuoespatial, visuoperception, praxis, and executive functions, including the following tests: Temporal, Spatial and Personal Orientation; Digit spans (forwards and backwards), Block Design (abbreviated so that items 6 to 9 were scored only for accuracy (1 point) without a time bonus) and Similarities (abbreviated to the first 10 items) subtests of Wechsler Adult Intelligence Scale-Third Edition (WAIS-III); The Word List Learning test from the Wechsler Memory Scale-Third Edition (WMS-III) (without using the interference list); Repetition (2 words and 2 sentences); Verbal comprehension (to correctly execute 2 simple, 2 semi-complex and 2 complex commands extracted from the ADAS-cog and the Barcelona test battery; the abbreviated 15-item Boston Naming Test; the Poppelreuter test; Luria's Clock test; The 15-Objects test; the Automatic Inhibition subtest of the *Syndrom Kurtz Test* (SKT); Phonetic Verbal Fluency (words beginning with 'P' in one minute); Semantic Verbal Fluency ('animals' in one minute); and the Spanish version of the Clock Test.

DNA extraction, SNP selection and genotyping

DNA from 1245 MCI samples was extracted using commercial methods. SNP selection was based on a review of the literature. Here, only those SNPs in loci identified by GWAS or meta-GWAS efforts were selected. Molecular genotyping methods are described elsewhere.(Lacour et al., 2016)

Statistical Analysis

Statistical analysis was performed using commercially available software (SPSS for Windows, IBM SPSS Statistics v. 20). To compare demographic, clinical, and genetic data among the four groups, an Analysis of Variance (ANOVA) or a Chi square were carried out for the quantitative and qualitative variables, respectively. To compare neuropsychological performances on the NBACE tests at baseline an Analysis of Covariance (ANCOVA), adjustments for gender, age, and education level were made. When a variable was statistically significant, Bonferroni *post-hoc* multiple comparisons were performed. To investigate the association between neuropsychological endophenotypes and genetic markers, they were stratified into five MCI phenotype related conditions, the four MCI phenotypes (amnestic/ non-amnestic x probable / possible) and the MCI total sample. A multivariate linear regression analysis was performed using PLINK (Purcell, 2010). The PLINK software also analyzed the genetic variations by calculating the allelic frequency in our population, the Hardy-Weinberg equilibrium, deviations of the allelic frequency between the study groups using the Chi test or the Fisher's exact test, and linkage desequilibrium (LD) with flanking markers. When a variable was statistically significant,

Bonferroni multiple comparison correction was calculated following this simplified formula: [*5MCI phenotypes related conditions (including MCI total sample) x 8 cognitive domains x 41 snps (including APOE)/0.05=10l-E*$^{-5}$]. Thus, only observed associations below $p \leq 10^{-5}$ were selected. The trend towards association was assumed at $p \leq 10^{-4}$.

The graphical representation of data were carried on through Heat Maps, where the individual p-values values contained in a matrix were represented as color-coding representing the statistical significance values taken by snps and the hierarchically Neuropsychological endophenotypes through The R- The Project R for Statistical Informatics software version 3.3.1 (http://www.r-project.org/).

Ethical Issues

The study protocol complied with national legislation and the Code of Ethical Principles for Medical Research Involving Human Subjects of the World Medical Association. Written informed consent was obtained from all participants prior to blood extraction. IRB approval was also obtained for this specific project (PI13/02434).

Results

Data from 1245 MCI individuals were classified into four MCI phenotypes related conditions: Pr-aMCI (*n*= 262; 21.0%), Pss-aMCI (*n*= 549; 44.1%), Pr-naMCI (n= 76; 6.1%) and Pss-naMCI (n= 358; 28.8%). In the whole sample, most of the MCI patients displayed a multiple domain impairment (n= 1223; 98.2%) whereas single domain affection

appeared only in 22 subjects (n= 22; 1.8%). In amnestic MCI individuals, storage pattern of memory impairment was observed in 563 subjects (69.4%) and retrieval impairment was found in 248 subjects (30.6%). Although no statistically significant differences among MCI groups were found in age and sex, they did significantly differ in educational level. All the subsequent analyses were consequently adjusted for education (Table 1).

Insert Table 1 About Here

Confirming our previous observation, we observed in the MCI subtypes that the *APOE*-ε4 allele showed significant difference in distribution with the following order: Pr-aMCI>Pss-aMCI>Pss-naMCI>Pr-naMCI subtypes (Espinosa et al., 2013). With regard to clinical variables, Pr-aMCI and Pr-naMCI subjects had lower Hachinski Ischemia Scale scores than Pss-aMCI and Pss-naMCI groups (see Table 1).

In our analysis, several neuropsychological variables showed statistically significant differences among groups (see Table 2). However, Global orientation, Learning, Delayed recall and Recognition memory of the WMS-III survived Bonferroni's correction for the amount of test performed.

Insert Table 2 About Here

In the MCI total sample, the *APOE*-ε4 allele, the strongest risk factor for AD, showed a significant effect on all three endophenotypes of the WMS-III: Learning (β=-1.35, $p=2.91x10^{-6}$), Delayed Recall (β=-0.76, $p=4.1x10^{-10}$) and Recognition (β=-0.58, $p=9.67x10^{-5}$) (see Table 3.A and Figure 1). The *APOE*-ε4 influenced also Learning (β=-1.37, $p=5.86x10^{-5}$) in Pss-aMCI (Table 3.B).

Insert Table 3 About Here

Insert Figure 1 About Here

Of note, the Delayed Recall effect of *APOE*-ε4 on the entire MCI sample was additionally supported by a SNP on strong Linkage disequilibrium with *APOE*, rs2075650 (*TOMM40* locus, β=-0.64, p=9.159E-06, Table 3.A and Figure 1 (for more details, see supplementary data available at: http://detritus.fundacioace.com/pub/supp_data/). We also detected nominal association with other susceptibility AD SNPs and endophenotypes in the entire MCI sample (Figure1). Thus, *APOE*-ε4 allele showed nominal association with Temporal orientation (β= -0.16, p=0.0015). For a SNP on the *CLU* locus (rs9331888), we found association with Delayed Recall (β= -0.31, p=0.006) and Verbal Comprehension (β=-0.07, p=0.003).

When we looked at the MCI subgroups, the *HS3ST1* locus (rs6448799) showed association with Backward Digits (β=0.52, p=7.57E-05) in the Pr-aMCI group (Table 4 and Figure 2). In addition, we detected a significant association between Repetition and the SNP on *AP2A2* locus (rs10751667) in the Pr-aMCI group (β=- 0.19, $p=5.34x10^{-6}$, Table 4 and Figure2). All these association survived Bonferroni correction (for more details, see supplementary data available at: http://detritus.fundacioace.com/pub/supp_data/).

Insert Table 4 About Here

Insert Figure 2 About Here

Discussion

This work investigated the association between 41 AD-related loci and cognitive domains in a sample of 1250 MCI subjects. To our best knowledge this is one of the largest MCI dataset explored to establish correlations between novel AD loci and MCI neuropsychological endophenotypes. Specifically, what we have explored whether different AD candidate loci could be quantitative trait loci in MCI baseline cognitive endophenotypes considered as a whole clinical paradigm or stratified into four clinical phenotypic-conditions (Espinosa et al., 2013). However, the MCI total sample Heat Map,

can be interpreted as the "big picture" in the genetics effects on neuropsychological metrics in this MCI population. Importantly, in the whole MCI sample, the only statistical significant observation was the *APOE*-ε4 locus. This observation is in agreement with previous findings in the literature, because, the *APOE*-ε4 allele is a well-established risk factor previously reported for the development of AD (Bennett et al., 2003; Corder et al., 1993; Evans et al., 2003; Wilson et al., 2002) , and MCI pheno-conversion to AD (Elias-Sonnenschein et al., 2011; Lacour et al., 2016).

We found that, *APOE*-ε4 was clearly associated to memory function, that is, Learning, Delayed recall and Recognition endophenotypes on the WMS-III for the whole MCI sample. Other neuropsychological endophenotypes, such as Global orientation, Attention and working memory, Executive functions, Language, Praxis and Visual gnosis there were not found statistically significant related to *APOE*-ε4. Our results are according to previous studies that reported that the presence of ε4 allele was strongly associated to greater memory dysfunction in MCI patients who were near to AD dementia conversion (Albert et al., 2007; Boyle et al., 2010; Farlow et al., 2004; Petersen et al., 1995; Smith et al., 1998; Wilson et al., 2002) and from cross-sectional findings that suggested the ε4 allele is associated with the level of memory dysfunction among persons with MCI (Bartres-Faz et al., 2001; Ramakers et al., 2008). Moreover, we reported previously the effect of presence of at least one *APOE*-ε4 allele at baseline, on Delayed Recall neuropsychological performance among Pr-aMCI, Pss-aMCI, Pr-naMCI and Pss-na MCI groups (Espinosa et al., 2013). Not surprisingly, the presence of ε4 allele was associated with all three endophenotypes of the WMS-III, Learning, Delayed Recall and Recognition of the WMS-III.

Regarding the association between the presence of ε4 allele and learning for the Pss-aMCI phenotype, that is, for those MCI subjects with memory impairments and presence of comorbidities such as anxiety/ depression or cerebrovascular disease, that could explain their cognitive deficits , our results are not according to a previous study (Mackin et al., 2013) that found a higher frequency of ε4 allele carriers in MCI patients with subsyndromal symptoms of depression , but not associated with a poorer cognitive functioning on Alzheimer's Disease Assessment Scale (ADAS)(Rosen et al., 1984).

Of note, there are no other statistical significant associations observed in the complete MCI dataset. This lack of findings can be interpreted potentially as a real lack of power of the present dataset for detecting very small effects. Maybe the AD risk loci identified to date have only a subtle effect in MCI endophenotypes. If true, real effects of selected loci could be only detected using larger series or via meta-analyses as previously occurred in case-control studies looking for AD risk genes (Lambert et al., 2013). This interpretation must be corroborated using larger series and subsequent meta-analyses in the future.

The *TOMM40* was associated only with Delayed recall on memory function for the MCI total sample. A previous study(Cervantes et al., 2011) showed that the genetic variation in *APOE* cluster region, *TOMM40* single nucleotide polymorphisms (Roses, 2010; Roses et al., 2010) was associated with progression from MCI stage to AD (rs59007384 and rs11556510), as well as with a shorter time to progression from MCI status to AD (rs10119), although these results could not be replicated in independent series. Interestingly, The *TOMM40* and *APOE* has been previously reported (Caselli et al., 2009) as significantly influenced age-related memory performance, but appear to do so

independently of each other. Then, when the *APOE*-ε4 is not included in the analysis, *TOMM40* appears as a factor of risk associated to worst performances on Delayed Recall for the MCI total sample. Accordingly, we detected some associations for rs2075650-*TOMM40* but closely resembling *APOE-ε4* findings. However, conditional analysis using epsilon 4 carrier status was conducted for rs2075650-*TOMM40* and null significance ($p>0.05$) was obtained for all endophenotypes under study. This analysis suggests that associations detected for *TOMM40* were strictly related to its linkage disequilibrium with the *APOE* locus in this dataset. Regarding MCI subtypes, The *HS3ST1* locus was found associated as a protective factor with Backward Digits better performances on working memory function for Pr-aMCI phenotype, that is, for those MCI patients with memory impairment and without comorbidities (i.e anxiety/depression or cerebrovascular disease) that could explain their cognitive deficits(Espinosa et al., 2013; Lopez et al., 2007, 2003; Petersen et al., 1999; R. C. Petersen, 2004). Interestingly, the *HS3ST1* is a new AD locus recently reported (Desikan et al., 2015) and its gene expression was found altered in AD compared with control brains. The previous study also demonstrated a genetic overlap between AD, C-reactive protein (CRP), and plasma lipids (i.e triglycerides (TG), high-(HDL) and low-density lipoprotein (LDL) levels). Importantly, working memory impairments becomes more pronounced during MCI and also, at very early stages of AD is marked by working memory impairments as well as by executive dysfunction and to episodic memory deficits. These cognitive deficits begin during MCI stage and appear to be a sign of progression to AD.(Kirova et al., 2015). The observed protective effect of *HS3ST1* preserving working memory in Pr-aMCI phenotype observed in the present study independently support the role of this locus in AD pathogenesis. However this finding has

absolute novelty and requires independent confirmation in other series. Hence, the results must be interpreted it cautiously.

Finally, *AP2A2* was found associated to Repetition on language functions worst performance for Pr-aMCI phenotype. Remarkably, recent proteomic analysis identified dysfunction in cellular transport, energy, and protein metabolism in different brain regions of atypical frontotemporal lobar degeneration (FTLD) with fused in sarcoma inclusions (aFTLD-U) related to *AP2A2* (Martins-De-Souza et al., 2012). This finding is notorious and point to the notion that some AD candidate loci might be actually involved in other neurodegenerative disorders not related to AD pathogenesis itself. In fact there are some evidences in the literature suggesting the existence of cross-contaminations of FTLD cases in AD datasets and vice versa (Hernández et al., 2014). Further studies are necessary to corroborate this preliminary finding. The isolation of multiple genetic loci associated to other cognitive disorder is necessary to deeply evaluate this possibility. Moreover, there is a big fraction of missingness (n=152) probable due to technical problems during the genotyping.

Our study suggests that there are not major effects of AD risk genes on neuropsychological endophenotypes measured in MCI subjects. However this conclusion must be taken very cautiously due the existence of important limitations in the present study. One of the limitations is due to the design. The approach is a cross-sectional study using baseline data, maybe longitudinal recording of cognitive decline might be more sensitive to detect the effects of AD risk loci in cognitive decline. It should be also taken into account the modest sample size of MCI subjects compared to previous studies focused on AD (Lambert et al.,

2013; Ruiz et al., 2014). This fact could explain lack of significance in SNPS, like *CLU*, previously reported in our meta-analysis as potential markers for MCI to AD progression(Lacour et al., 2016) however in this study only showed tentative additional association signals in the whole MCI sample. Additionally, we were not able to measure sensitivity and specificity values because of the smalls sample sizes. However, compared to case controls status, QTLs can offer increased statistical power and thus have reduced requirement on sample size.(Shen et al., 2014). Another limitation is the lack of replication in independent series. However, previous reported QTL analyses (Hu et al., 2011; Shen et al., 2014) , with relatively modest sample sets and without replication studies have found some significant genetic markers .

In summary, the present data support that as in previous studies, the role of *APOE* genotype in episodic verbal memory for the amnestic MCI phenotypes and for the MCI whole sample, and failing to identifying other associations with top AD risk genes. Furthermore, in our study the *HS3ST1* was found associated to Backward Digits on working memory function for the Pr-aMCI phenotype. Other locus as *AP2A2* was associated to language functions on Repetition only for the Pr-aMCI phenotype, both evidences are supporting independently the involvement of these loci in adult neurodegenerative disorders. Further studies in larger longitudinal MCI samples are now warranted to replicate these data, and to disentangle the genetic factors that influence the MCI endophenotypes. Information on loci acting in different cognitive domains associated to MCI will be of relevance for mapping refinements of risk loci and for the selection of entry points for drug development in AD.

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Table 1. Comparison of demographic, clinical and *APOE*-ε4 data between groups.

	Pr-aMCI	Pss-aMCI	Pr-naMCI	Pss-naMCI	Statistics	p
N (%)	262 (21.0)	549 (44.1)	76 (6.1)	358 (28.8)		
Sex n (%) Female	169 (64.5)	354 (64.5)	42 (55.3)	245 (68.4)	5.12_1	.163
Education in years (n (%))					17.87_1	.001
< 8	192 (73.3)	446 (81.2)	47 (61.8)	280 (78.2)		
> 8	70 (26.7)	103 (18.8)	29 (38.2)	78 (21.8)		
Age in years (mean/SD)	76.5/6.4	76.2/7.3	76.3/5.7	75.2/7.4	2.21_2	.085
MMSE (mean/SD)	24.6/3.0	25.1/3.0	26.8/2.0	27.0/2.3	54.66_2	.001
HIS (mean/SD)	1.9/1.3	2.8/2.1	2.7/3.8	3.0/2.7	11.12_2	.001
APOE-ε4 n (%) (presence of ε4 or ε4 ε4)	119 (45.4)	175 (32.0)	15 (19.7)	94 (26.3)	32.04_1	.001

Pr-aMCI: Probable amnestic Mild Cognitive Impairment; Pss-aMCI: Possible amnestic Mild Cognitive Impairment; Pr-naMCI: Probable non- amnestic Mild Cognitive Impairment; Pss-naMCI: Possible non-amnestic Mild Cognitive Impairment. MMSE: Mini-Mental State Examination; HIS: Hachinski Ischemia Scale; SD: Standard deviation; $_1$: χ^2; $_2$: F.

Table 2. The ANCOVA comparing neuropsychological domains scores between MCI phenotypes

Cognitive domains on NBACE	Pr-aMCI M (SD)	Pss-aMCI M (SD)	Pr-naMCI M (SD)	Pss-naMCI M (SD)	F (3, 537)	Eta^2	Multiple Comparisons with Bonferroni'scorrection Pr-aMCI vs.		
							Pss-aMCI	Pr-naMCI	Pss-naMCI
Global orientation	13.60 (1.66)	13.88 (1.44)	14.16 (1.23)	14.64 (.78)	37.41	.08	.032	.007	.001
Attention and working memory									
Forward Digits	6.63 (1.74)	6.57 (1.68)	6.83 (1.55)	6.84 (1.72)	2.28	.01	NS	NS	NS
Backward Digits	3.36 (1.52)	3.14 (1.50)	3.46 (1.66)	3.71 (1.55)	11.88***	.03	NS	NS	.015
Executive functions									
SKT (time in seconds)	42.90 (16.38)	46.42 (22.40)	42.28 (20.07)	39.04 (13.34)	11.74***	.03	NS	NS	NS
SKT (errors)	4.69 (5.35)	4.59 (5.17)	3.37 (3.86)	3.00 (3.72)	10.49***	.03	NS	NS	.001
PVF	9.01 (4.40)	8.14 (4.04)	9.84 (4.45)	10.03 (4.37)	16.75***	.04	.030	NS	.013
SVF	10.89 (3.97)	11.18 (3.77)	12.55 (4.21)	13.50 (4.16)	32.78***	.07	NS	.007	.001
Similarities WAIS-III	7.64 (3.18)	7.95 (2.88)	9.04 (2.86)	9.00 (2.60)	18.21***	.04	NS	.001	.001
Language									
Visual naming (15-BNT)	12.33 (2.67)	12.69 (2.30)	13.33 (1.62)	13.55 (1.77)	19.50***	.045	NS	.003	.001
Verbal Learning and Memory WMS-III									
Learning (Trials 1+2+3+4)	15.51 (5.14)	16.84 (4.72)	22.08 (5.11)	23.53 (5.04)	199.90***	.33	.001	.001	.001
Delayed Recall	.85 (1.14)	1.59 (1.50)	4.80 (1.73)	5.33 (1.77)	627.76***	.60	.001	.001	.001
Recognition memory	17.72 (2.88)	19.10 (2.56)	21.88 (1.58)	21.99 (1.65)	209.38 ***	.34	.001	.001	.001
Praxis									
Block Design	2.63 (1.38)	2.64 (1.39)	3.07 (1.14)	3.03 (1.21)	8.71***	.02	NS	NS	.001
Imitation	2.81 (1.25)	2.89 (1.21)	3.20 (1.05)	3.27 (1.04)	11.03***	.03	NS	NS	.001
Visual gnosis									
Poppelreuter test (responses)	8.72 (1.55)	8.87 (8.85)	9.27 (1.00)	9.31 (1.09)	13.02***	.03	NS	.008	.001
Luria's Clocks test	2.43 (1.26)	2.54 (1.21)	2.97 (1.04)	2.83 (1.04)	9.35***	.02	NS	.002	.001
The 15-Objects test	10.70 (2.45)	10.79 (2.77)	11.40 (2.06)	11.48 (2.07)	1.70	.02	NS	NS	NS
Global Cognition Clock Test	4.56 (2.28)	4.90 (2.11)	5.48 (1.78)	5.71 (1.75)	20.11***	.05	NS	.004	.001

Global orientation: summatory of Temporal+Spatial+Personal orientations; WMS-III: Wechsler Memory Scale, Third Edition; The abbreviated BNT: Boston Naming Test with 15 visual items; Recognition memory: correct answers; Block Design: WAIS-III; SKT: Automatic Inhibition Symdrom Kurztest; PVF: Phonemic verbal fluency; SVF: Semantic verbal fluency ; WAIS-III: Weschler Adult Intelligence Scale, Third edition; M: Mean; S.D: Standard deviation; NS: $p \geq .05$; *$p < .05$, **$p \leq .005$, ***$p \leq .001$;‡: It was practically a constant.

Table 3. Major results of AD loci linked to *APOE* in MCI endophenotypes by clinical subtypes.

Endophenotypes	**N**	**Locus/ snp**	β	**L95**	**U95**	**p-value**
3.A MCI Total sample						
Learning	**1243**	***APOE-ε4***	-1.35	-1.95	-0.76	2.91E-06*
Delayed Recall	**1243**	***APOE-ε4***	-0.76	-1.00	-0.51	4.10 E-10*
	1137	***TOMM40*-rs2075650**	-0.64	-0.92	-0.34	9.26 E-06*
Recognition	**1243**	***APOE-ε4***	-0.58	-0.88	-0.27	9.77 E-05*
3.B Pss-aMCI						
Learning	**549**	***APOE-ε4***	-1.37	-2.07	-0.68	5.82 E-05*
Delayed Recall	**549**	***APOE-ε4***	-0.36	-0.59	-0.12	0.01
Recognition	**549**	***APOE-ε4***	-0.43	-0.85	-0.02	0.03

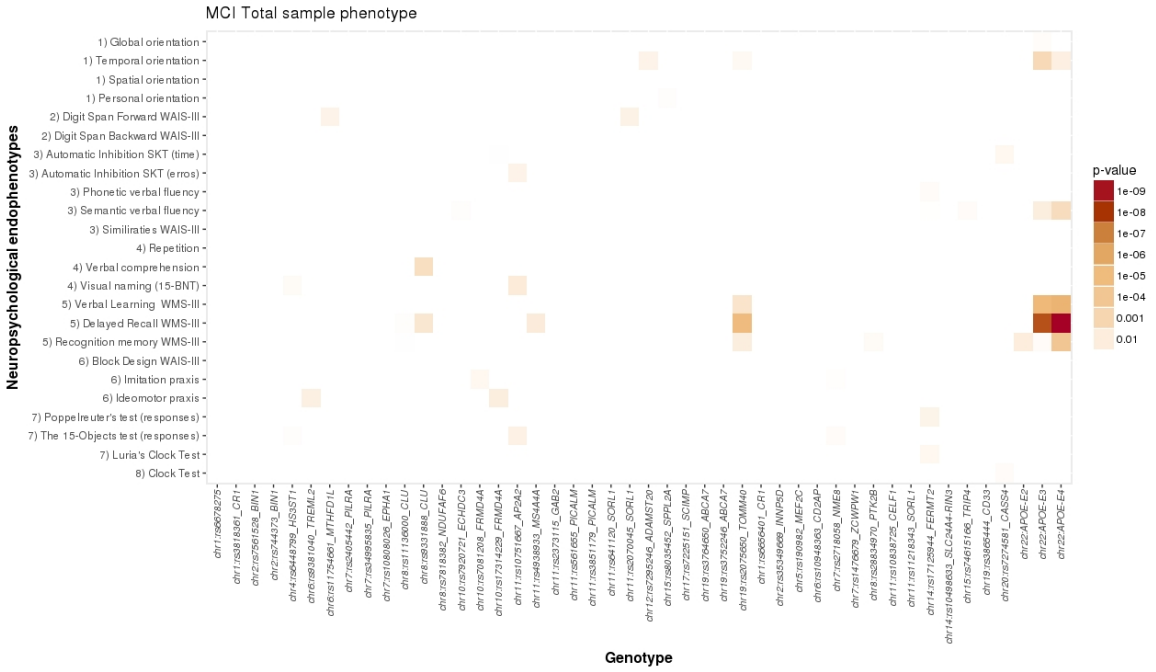
β: Beta; L-U95: confidence intervals 95% ; *Statistically significant after Bonferroni's correction ($p \leq 10\text{-}E^{-5}$).

Table4. Major results of AD loci unlinked to *APOE* in MCI endophenotypes by clinical subtypes.

Endophenotypes	**N**	**Locus/ snp**	**β**	**L95**	**U95**	**p-value**
Pr-aMCI						
Backward Digits	262	***HS3ST1*-rs6448799**	0.52	0.25	0.78	7.57 E-05*
Repetition	110	***AP2A2*-rs10751667**	- 0.19	- 0.27	- 0.11	5.34 E-06*

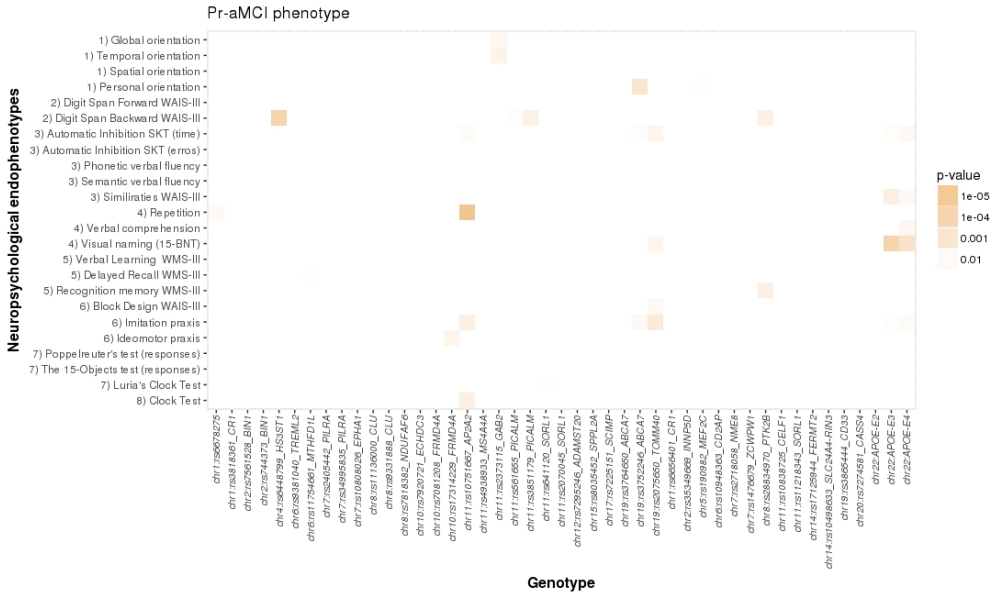
β: Beta; L-U95: confidence intervals 95% ; *Statistically significant after Bonferroni's correction ($p \leq 10\text{-}E^{-5}$).

Figure1. MCI Total Sample Heat Map



1)Orientation; 2)Attention and working memory; 3)Executive functions; 4) Language; 5)Verbal Learning and Memory; 6)Praxis; 7) Visual gnosis; 8) Global Cognition; Global orientation, summary of Temporal + Spatial + Personal orientations; WAIS-III: Wechsler Adult Intelligence Scale, Third edition; SKT: Syndrom Kurz Test; 15-BNT: the abbreviated Boston Naming Test with 15 items; Verbal learning WMS-III = 1st+2nd+3rd+4thtrial scores; WMS-III: Wechsler Memory Scale, Third Edition.

Figure 2. Pr-aMCI Heat Map



1)Orientation; 2)Attention and working memory; 3)Executive functions; 4) Language; 5)Verbal Learning and Memory; 6)Praxis; 7) Visual gnosis; 8) Global Cognition; Global orientation, summary of Temporal + Spatial + Personal orientations; WAIS-III: Wechsler Adult Intelligence Scale, Third edition; SKT: Syndrom Kurz Test; 15-BNT: the abbreviated Boston Naming Test with 15 items; Verbal learning WMS-III = 1st+2nd+3rd+4thtrial scores; WMS-III: Wechsler Memory Scale, Third Edition.

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7. DISCUSIÓN GENERAL

El objetivo general de esta tesis se fundamentó en contribuir al conocimiento sobre el DCL, centrado en la búsqueda de los factores de riesgo neuropsicológicos, de neuroimagen y genéticos, predictores de conversión a demencia, principalmente EA. Incluimos endofenotipos neuropsicológicos que han demostrado su utilidad en la detección de la fase prodrómica (DCL) y leve de la EA tales como los perfiles de memoria episódica verbal;[59,73,120,121,142] biomarcadores de neuroimagen en la investigación de la EA prodrómica[52,53] o del DCL debido a EA[4] como la RM estructural, el PET-FDG y el PET-PIB relacionados con la neurodegeneración y depósito de βA; marcadores genéticos pre-GWAS, como *APOE*-ε4[41] e integrando los nuevos conocimientos obtenidos de la investigación de los estudios genéticos GWAS o meta-GWAS en los pacientes con EA.[100] Además, investigamos la relación de los genes asociados a la EA en los endofenotipos neuropsicológicos de interés y su correlación con diferentes fenotipos clínicos del DCL.

Aunque algunos estudios, derivados del *Cardiovascular Health Study (CHS) Cognition Study*,[102,103,66] han utilizado la clasificación de DCL-a-Pr y DCL-a-Ps la clasificación en cuatro fenotipos clínicos incluyendo el DCL-na (amnésico/no amnésico x probable/posible) se utiliza por primera vez en nuestro trabajo. En nuestro conocimiento, ningún estudio previo [102,103, 66] había utilizado en su clasificación del DCL los subtipos no amnésico probable y posible, ni tampoco existían estudios con baremos o puntos de corte para su población diana. Asimismo, a nivel metodológico, la cohorte de sujetos con DCL del *CHS* fue reclutada de la población general,[66] mientras que en nuestro trabajo se reclutó de la población clínica de DCL y en un único centro, la Unidad de Diagnóstico en Fundació ACE.[24] De acuerdo a Brodaty et al.[29] el procedimiento de selección de la muestra de DCL debe tenerse en cuenta en la

interpretación de los resultados, ya que la muestra de sujetos con DCL de la población general, rinde mejor en las pruebas de memoria y tienen menos posibilidades de ser portadores del alelo *APOE*-ε4 que los provenientes de una población clínica.

Los resultados obtenidos en nuestro trabajo, han demostrado que el fenotipo DCL-a-Pr a nivel de almacenamiento, tiene el mayor riesgo de conversión a demencia, principalmente EA, en comparación al resto de fenotipos de DCL (amnésico posible, no amnésico probable y no amnésico posible, respectivamente). Concretamente, el subtipo DCL-a-Pr a nivel de almacenamiento, obtuvo 8,5 veces más riesgo que el grupo DCL-na-Ps, el cual resultó tener la conversión más lenta a demencia. Acorde con estudios previos,[28,70] la alteración de memoria a nivel de almacenamiento (más frecuente en el subtipo DCL-a-Pr que en el DCL-a-Ps) está relacionado con un mayor riesgo de conversión a demencia en comparación a la alteración de la memoria de recuperación.

Sin embargo, el pronóstico en los subtipos con DCL-a-Pr y DCL-a-Ps es similar, acorde a un estudio previo,[103] así como para el subtipo DCL-na-Pr en nuestro trabajo. No ocurriendo así para el subtipo DCL-na-Ps en que tras un periodo de seguimiento medio de 26,6 meses y un rango de 6 a 68 meses, sólo convierten a demencia el 50% de los pacientes. Acorde a estudios previos, [52,61,164] con independencia del subtipo de DCL, la mayoría de los sujetos con DCL que convirtieron a demencia durante el seguimiento en nuestro trabajo (257/550, 46,7%) principalmente desarrollaron EA (117/257,45,5%), 51 (19,8%) DV, 50 (19,5%) DM, 25 (9,7%) DFT, 12 (4,7%) DCLewy y 2 (8%) Demencia por Enfermedad de Parkinson. No obstante, algunos estudios postulan que el DCL-na tiene un riesgo incrementado de conversión a demencia no-EA.[138,188]

Entre todos los subtipos de DCL de nuestra serie, se halló una mayor frecuencia de patrones de alteración múltiple dominio que único dominio, principalmente en el DCL-a-Pr, consistente con estudios previos,[102,126] pero no en todos.[141] Nuestros resultados sugieren que el DCL-a-ud tradicional [142] raramente se diagnostica cuando se administra una batería neuropsicológica exhaustiva, dado que se suelen encontrar otros déficits cognitivos cuando se amplía la evaluación neuropsicológica.[127] En nuestro trabajo, los endofenotipos neuropsicológicos asociados a conversión a demencia para la muestra global de 550 sujetos con DCL fueron Orientación a la Realidad (incluyendo la Orientación en tiempo, espacio y persona), el Recuerdo Diferido de la Lista de Palabras de la WMS-III y los Relojes de Luria.

Profundizar en el conocimiento sobre la relación entre los endofenotipos neuropsicológicos y los biomarcadores de neuroimagen, puede ayudar a identificar a los sujetos con DCL que convertirán a demencia tipo EA, que ya sufren la enfermedad en estadio prodrómico. Aunque en nuestro trabajo no dispusimos de biomarcadores de LCR si tenemos en cuenta que el objetivo del estudio retrospectivo fue detectar los sujetos con riesgo a desarrollar demencia, no sólo la EA, esta no fue una cuestión crucial. Además, el análisis de biomarcadores de LCR no era una técnica obligatoria en España cuando se llevó a cabo este proyecto. Incluso hoy en día, la punción lumbar no es mandatoria para el diagnóstico de DCL en la práctica clínica en nuestro país. En cambio, en nuestro trabajo, dispusimos de biomarcadores de neuroimagen para la investigación del fenotipo DCL-a-Pr a nivel de almacenamiento y encontramos que únicamente un peor rendimiento en el *Composite* Cognitivo Recuerdo Diferido en Memoria se halló relacionado con una mayor carga de βA mediante PET-PIB, un menor volumen hipocampal en RM y un menor metabolismo en PET-FDG.

Sin embargo, los otros endofenotipos neuropsicológicos (el rendimiento en los *Composites* Cognitivos de Aprendizaje, Funciones ejecutivas, Lenguaje y Praxias) no se hallaron relacionados con los biomarcadores de neuroimagen utilizados. Estos resultados son consistentes con estudios previos [36,42,75,111,117,158,190] que han sugerido que un peor rendimiento en el Recuerdo Diferido está relacionado con un menor volumen hipocampal y un hipometabolismo cerebral en pacientes con DCL próximos a convertir a demencia tipo Alzheimer. Además, la replicación de nuestros resultados en la base de datos de ADNI para el PET-PIB confirmó que el Recuerdo Diferido es el endofenotipo neuropsicológico óptimo para detectar la EA prodrómica, en comparación con los otros dominios cognitivos.

Los sujetos con DCL-a-Pr a nivel de almacenamiento muestran una alta homogeneidad fenotípica y contiene algunos miembros con un patrón cognitivo y de biomarcador, estrechamente similar a la EA, por lo que es probable que conviertan en un futuro próximo. Además, el enriquecimiento observado del alelo *APOE*-ε4 (70,0%), en el estudio con biomarcadores fue muy similar al de las series histopatológicas publicadas en la EA.[125] De hecho, el seguimiento de estos pacientes mostró que casi todos ellos (17/20, 85%) convirtieron a demencia, principalmente tipo EA (15/17, 88,2%) y sólo 2 (11,8%) DM (EA con enfermedad cerebrovascular asociada).

Mientras que algunos de ellos pudieron encontrar representación en el modelo fisiopatológico actual de la EA propuesto por Jack et al.,[83,84,86,87] cuya hipótesis es que la βA del cerebro se acumula precozmente, seguido por cambios en los marcadores del metabolismo neuronal y la atrofia cerebral, otros o bien mostraron atrofia hipocampal e hipometabolismo sin amiloidosis clínica [81,83,84,86,87] o bien, una alta

carga de βA sin suficiente atrofia del hipocampo o hipometabolismo cerebral. [83,84,86,87]

Dos de los sujetos del grupo DCL-a-Pr a nivel de almacenamiento no se hallaron en ninguno de los tres diferentes conjuntos de modelos de biomarcadores, [83,84,86,87] ya que aunque ambos desarrollaron demencia tipo EA, se encontraban en los límites para ser consideraros con alta carga de βA en base al VHa en la RM y del SUVR del PET-FDG. Este hallazgo fue inesperado puesto que estudios previos [144,191] con DCL encontraron que el patrón de biomarcador central proporcionaba evidencia clínica del modelo de EA en pacientes con DCL. Aunque todos los pacientes fueron positivos a nivel basal en al menos un biomarcador de neuroimagen, sólo 11 (55%) fueron positivos para los tres biomarcadores. Sin embargo, con respecto al endofenotipo neuropsicológico, todos obtuvieron un rendimiento alterado en la puntuación del *Composite* Cognitivo Recuerdo Diferido en Memoria y, lo que es más importante, prácticamente todos convirtieron a demencia, principalmente EA. Para una gran fracción de nuestros sujetos con DCL-a-Pr a nivel de almacenamiento, el fenotipo clínico y endofenotipo neuropsicológico precedieron a los biomarcadores de amiloide y neurodegeneración. Sin embargo, serían necesarios más estudios, con una mayor muestra de sujetos con DCL y más heterogénea, así como datos anatomopatológicos disponibles para corroborarlo.

Respecto a la distribución de los alelos de *APOE* en el DCL, acorde con estudios genéticos previos,[40] en nuestra serie de 550 sujetos con DCL el alelo más frecuente fue el ε3 (62,5%) y el menos frecuente el ε2 (8,0%). Cabe remarcar, que ninguno de los 550 sujetos con DCL de nuestro estudio tenía el haplotipo ε 2 ε 2, el cual se ha

relacionado con la protección contra la EA de inicio tardío.[41] La frecuencia del alelo *APOE*-ε4 fue significativamente mayor en el DCL-a-Pr (53,9%) que en el resto de subtipos de DCL (DCL-a-Ps: 26,9%, DCL-na-Pr: 16,2%, DCL-na-Ps: 27,0%).

El seguimiento longitudinal de los 550 sujetos con DCL mostró que, el grupo DCL-a portador de ε4 obtuvo una probabilidad 1,7 veces mayor de desarrollar la EA que el DCL-a no-portador y el grupo DCL-na portador de ε4 obtuvo un riesgo 1,2 veces mayor de desarrollar la EA que el DCL-na no-portador. Estos resultados han sido confirmados en meta-análisis posteriores como en nuestro meta-análisis con cuatro series independientes de DCL y otros estudios independientes, [98,178] donde se demostró el efecto protector del alelo ε2 contra la fenoconversión de DCL a EA y por el contrario, el efecto de riesgo del alelo ε4.

Hasta la fecha, sólo el locus *APOE* ha mostrado un efecto coherente sobre el DCL y su progresión a EA. En la literatura previa [32,55,159] encontramos diversos estudios en sujetos con DCL usando marcadores obtenidos de GWAS previos, pero sin meta-análisis, o bien que sólo habían investigado el poder predictivo de *APOE*-ε4.[55] Algunos estudios previos,[3,147,172] investigaron los factores de riesgo genéticos GWAS en la fenoconversión del DCL a la EA partiendo de una serie poblacional y no una serie clínica de DCL como en nuestro trabajo, en el que investigamos el papel de los marcadores genéticos establecidos de la EA (tipo SNP) derivados de GWAS en la progresión de DCL a EA usando los datos de seguimiento de cuatro series independientes de sujetos con DCL (amnésico y no amnésico): las dos series alemanas AgeCoDe y DCN, ACE, en Barcelona, España y la ADC en Ámsterdam. En nuestro conocimiento, éste es el primer meta-análisis sistemático realizado con marcadores

genéticos de la EA en sujetos con DCL para investigar la fenoconversión a EA y con la muestra más amplia de sujetos con DCL publicada (n= 3.326) confirmando la asociación de *APOE*-ε4 como factor de riesgo firmemente establecido con la progresión de DCL a EA (*APOE*-ε4 (alelo rs429358 C). Este resultado confirma el de estudios previos, [98,178] que realizaron meta-análisis con seguimiento longitudinal, pero con muestreo poblacional y centrados únicamente en el locus *APOE*-ε4. Específicamente, los portadores de los haplotipos ε4/ ε4 convirtieron en mayor número y más rápido a EA, que los portadores de los haplotipos, ε3/ ε4, ε3/ ε3 o ε2 ε4, ε2/ ε3, respectivamente, observándose un incremento del efecto de ε4 con la edad y alcanzando su efecto más pronunciado sobre la fenoconversión concentrado entre los 65 y 80 años. Por último, ningún portador del haplotipo ε2/ ε2 convirtió a demencia, confirmando su papel protector.

Aunque la EA posee una alta heredabilidad, que puede llegar hasta el 69%,[189] la capacidad predictiva de demencia tipo EA de los nuevos factores de riesgo genéticos identificados en la EA no se conoce adecuadamente. Los estudios GWAS y de secuenciación de exomas han revelado diversas variantes de baja penetrancia comunes o raras [13,23,44,74,78,79,90,100,123,167,172,173] y recientemente el consorcio IGAP ha identificado 11 nuevos loci asociados a la EA. [100] En relación a otros marcadores genéticos disponibles en la EA, los estudios publicados sobre la progresión del DCL a demencia tipo EA utilizando polimorfismos de un solo nucleótido (SNP), o combinaciones de SNPs en puntuaciones combinadas de genotipos (PGS), habían generado resultados contradictorios.[80,159] Hemos analizado todos los marcadores para LOAD disponibles, los loci conocidos del GWAS, tales como *CR1*, *BIN1*, *CD2AP*

EPHA1, CLU, MS4A4A, PICALM, ABCA7, CD33 y nueve de los once nuevos loci identificados por IGAP, [100] tales como *PTK2B, SORL1, SLC24A4-RIN3, INPP5D, MEF2C, NME8, ZCWPW1, FERMT2 y CASS4.* Se halló el locus que codifica para Clusterina (*CLU* rs9331888) como un factor genético independiente asociado a la conversión de DCL a EA. Estos resultados confirman los previamente hallados en *CLU* en dos estudios con sujetos con DCL.[32,159] Específicamente, la ausencia del alelo T del locus *CLU* se asoció a un mayor riesgo de fenoconversión a EA, de forma que los no-portadores del alelo T obtuvieron una mayor conversión (mayor número de conversores y una progresión más rápida) a demencia que los portadores, aunque el efecto fue mucho más sutil que el de *APOE*.

No obstante, en nuestro meta-análisis no se confirma una contribución importante del resto de loci identificados por IGAP, [100] ni de los previos, en la fenoconversión de DCL a EA. La puntuación combinada de genotipos (PGS1) que comprende nueve loci de riesgo de EA publicados con anterioridad al consorcio IGAP [100] (*CR1, BIN1, CD2AP, EPHA1, CLU, MS4A4A, PICALM, ABCA7 y CD33*) mostró sólo un pequeño efecto sobre el riesgo de progresión de DCL a EA en los portadores de *APOE*-ε4. Este hallazgo se ha confirmado en un trabajo independiente del consorcio CHARGE.[39] La ausencia de resultados positivos puede ser atribuible al tamaño de la muestra, el cual puede haber sido demasiado pequeño para poder detectar ciertas asociaciones. Además, sólo nueve de los once nuevos loci de riesgo encontrados por IGAP [100] estaban representados en PGS2 y PGS3 debido a problemas técnicos con ciertos marcadores como rs9271192 en *HLA-DRB5-HLA-DRB1* y para rs10838725 en *CELF1*. Por lo tanto, las conclusiones extraídas para PGS2 y PGS3 deben ser interpretadas con cierta cautela.

Usando la serie de DCL de Fundació ACE ampliada hasta 1.250 sujetos, nuestro trabajo de asociación entre endofenotipos neuropsicológicos y marcadores genéticos, confirmó la asociación del locus *APOE*-ε4 con el endofenotipo memoria episódica verbal de la Lista de Palabras de la WMS-III en la muestra total de DCL, DCL-a y DCL-a-Ps. De acuerdo con estudios previos transversales,[16,149] estos resultados confirman que el alelo *APOE*-ε4 está relacionado con el nivel de disfunción de la memoria en personas con DCL. Además, en nuestro trabajo retrospectivo y longitudinal, tener al menos un alelo ε4 del locus *APOE* se asoció con un peor rendimiento en el Recuerdo Diferido en los cuatro fenotipos clínicos de DCL (DCL-a-Pr, DCL-a-Ps, DCL-na-Pr y DCL-na-Ps). Sin embargo, aunque los endofenotipos neuropsicológicos que mejor predijeron conversión a demencia para nuestra amplia muestra retrospectiva con DCL fueron la Orientación a la Realidad (incluyendo Orientación en tiempo, espacio y persona), Recuerdo Diferido de la Lista de Palabras de la WMS-III y los Relojes de Luria, su efecto predictor a conversión resultó ser independiente a la presencia o ausencia de al menos un alelo ε4 del locus *APOE*.

Otros endofenotipos que encontramos relacionados con la fisiopatología de la EA corresponden a los Dígitos Inversos de la WAIS-III (función ejecutiva) y la Repetición verbal (lenguaje). Específicamente, en nuestro trabajo identificamos un efecto protector del locus *HS3ST1* (rs6448799) asociado a la memoria de trabajo y un efecto de riesgo del locus *AP2A2* (rs10751667) asociado a la Repetición verbal, en el fenotipo DCL-a-Pr. El efecto protector observado de *HS3ST1* asociado a un mejor rendimiento en la memoria de trabajo en el fenotipo DCL-a-Pr hallado en el presente estudio, apoya el papel independiente de este locus en la patogénesis de la EA. Sin embargo, este

hallazgo tiene novedad absoluta y requiere confirmación independiente en otras series. Por lo tanto, los resultados deben interpretarse con cautela.

El efecto observado en el marcador de *AP2A2* asociado a un peor rendimiento en la Repetición verbal en el fenotipo DCL-a-Pr podría estar relacionado con un reciente estudio sobre análisis proteómico que identificó disfunción en el transporte celular, la energía y el metabolismo de proteínas en diferentes regiones cerebrales de DLFT atípica con fusiones en inclusiones de sarcoma (DLFT-FUS) relacionadas con *AP2A2*.[108] Este hallazgo es notorio y apunta a la noción de que algunos loci candidatos de EA podrían estar realmente involucrados en otros trastornos neurodegenerativos no relacionados con la propia patogénesis de la EA. Algunos estudios sugieren la existencia de contaminaciones cruzadas de casos de DFT en diversas series de EA y viceversa. [77] Sin embargo, en nuestro estudio hay una gran fracción de sujetos que no lograron genotiparse para *AP2A2* probablemente debido a problemas técnicos durante su genotipado. Para corroborar este hallazgo son necesarios más estudios en series independientes.

Otro marcador encontrado en nuestro trabajo fue el locus *TOMM40* (rs2075650). Este marcador apareció significativamente asociado con el Recuerdo Diferido para la muestra total de DCL. Un estudio previo [34] mostró que la variación genética en la región del clúster *APOE*, en concreto, los SNPs *TOMM40* (rs59007384 y rs11556510) ,[162,163] se asociaron con una progresión más lenta de DCL a EA (rs10119). Estos resultados no pudieron ser replicados en series independientes. Los locus *TOMM40* y *APOE* habían mostrado previamente[33] estar influidos de forma significativa en el rendimiento en memoria asociado a la edad, pero parece que lo hacen de forma

independiente entre sí. En nuestro trabajo cuando *APOE*-ε4 no se incluye en el análisis, *TOMM40* aparece como un factor asociado a peores rendimientos en el Recuerdo Diferido para la muestra total de DCL. Por consiguiente, hemos detectado algunas asociaciones para rs2075650-*TOMM40* muy parecidas a los hallazgos en *APOE*-ε4. Sin embargo, realizamos un análisis condicional utilizando el estado portador de ε4 para rs2075650-*TOMM40* y se obtuvo una significación nula para todos los endofenotipos estudiados. Este análisis sugiere que las asociaciones detectadas para *TOMM40* estaban estrictamente relacionadas con su desequilibrio de unión con el locus *APOE* en nuestra población.

Además encontramos indicios de asociación adicionales tentativas, que no superaron la corrección de Bonferroni, para algunos SNPs en la muestra total de DCL. En particular, el alelo ε4 en el locus *APOE* se asoció con la Orientación temporal y el SNP (rs9331888) en el locus *CLU* se asoció con los endofenotipos Recuerdo Diferido y Comprensión verbal. Sin embargo, se debe tener en cuenta el tamaño modesto de nuestra muestra de DCL en comparación a otros estudios anteriores centrados en la EA. [100,167]Esto podría explicar la falta de significación de SNPs firmemente asociados a AD y conversión a demencia (p.ej. *CLU*) que fueron publicados previamente en nuestro meta-análisis como marcadores potenciales para la progresión de DCL a EA y que en nuestro estudio de asociación con endofenotipos sólo mostraron señales de asociación adicionales tentativas en el conjunto total de DCL.

En resumen, la presente tesis doctoral aporta a los estudios anteriores la caracterización del fenotipo clínico DCL-a-Pr a nivel de almacenamiento como el de mayor riesgo y la mayor tasa de conversión a demencia, principalmente EA, en comparación al resto de

fenotipos clínicos de nuestro trabajo. Tal y como reflejamos en la discusión de nuestro trabajo, en nuestro conocimiento, estos resultados se describen por primera vez en este estudio. Para este fenotipo clínico concreto de DCL, caracterizamos al endofenotipo neuropsicológico Recuerdo Diferido como el más óptimo para detectar a la EA prodrómica, en comparación con el resto de dominios cognitivos. Su validez se contrasta por su mayor correlación con los tres biomarcadores de neuroimagen disponibles en nuestro trabajo. Actualmente, la búsqueda de biomarcadores para identificar la EA en su fase prodrómica [51,53] o DCL debido a EA [4] se han centrado en métodos costosos y a menudo poco tolerados por los pacientes (por ejemplo, el PET). En cambio, las pruebas neuropsicológicas no son invasivas y pueden llegar a ser, en términos de coste-beneficio,[187] mejores predictores de la EA que las propias técnicas de neuroimagen.[54,71] Sin embargo, serían necesarios más estudios, con una mayor muestra de sujetos con DCL y más heterogénea, con biomarcadores en LCR así como datos anatomopatológicos disponibles para corroborarlo.

El meta-análisis sistemático en cuatro series independientes, de los marcadores genéticos de EA derivados de estudios GWAS en la fenoconversión de DCL a EA, no respalda una importante contribución de los nuevos loci de EA identificados por IGAP [100] en la evolución desde DCL a EA. Por lo tanto, concluimos que estos predictores genéticos no tienen valor clínico suficiente para identificar sujetos en riesgo de padecer EA. Tal y como reflejamos en la discusión de nuestro trabajo, este es el primer meta-análisis realizado en una serie clínica de DCL con los loci de riesgo de EA. Aunque esta muestra represente la serie más amplia publicada hasta la actualidad, pensamos que los efectos de los genes de EA en el proceso de conversión de DCL a demencia pueden ser muy pequeños. Esto implica que necesitaremos estudios con muestras más grandes para

verficarlos. Por el contrario, pudimos corroborar que el locus Clusterina (*CLU*) es un factor genético independiente asociado con la fenoconversión de DCL a EA. Este hallazgo tiene relevancia desde el punto de vista translacional porque coloca a la apolipoproteína J (codificada por el gen *CLU*) junto con el gen *APOE* como dianas terapéuticas para frenar la conversión a demencia.

Hallamos, además, otras correlaciones fenotipo-genotipo durante nuestro trabajo. En concreto, el fenotipo DCL-a-Pr se asoció una mayor frecuencia del alelo *APOE*-ε4 en comparación al resto de fenotipos clínicos de DCL, observándose un enriquecimiento del alelo *APOE*-ε4 (70,0%) en el DCL-a-Pr a nivel de almacenamiento muy similar al de las series histopatológicas publicadas en la EA.[125] Pudimos también constatar en el fenotipo DCL-a-Pr un efecto protector del locus *HS3ST1* (rs6448799) asociado a función ejecutiva y un efecto de riesgo del locus *AP2A2* (rs10751667) asociado al lenguaje. Estas correlaciones fenotipo-genotipo involucran a estos genes en la patogénesis de la EA y tienen novedad absoluta. Dada su novedad, estos hallazgos requieren confirmación independiente en otras series clínicas de DCL y necesitan ser corroborados en más estudios que relacionen múltiples loci genéticos con diferentes alteraciones cognitivas.

8. CONCLUSIONES

1. Tras el seguimiento de una amplia muestra de 550 sujetos con Deterioro Cognitivo Leve (DCL) el fenotipo DCL amnésico probable a nivel de almacenamiento demostró tener el mayor riesgo y la mayor tasa conversión a demencia. Específicamente, obtuvo 8,5 veces más riesgo de conversión a demencia, principalmente tipo EA, que el fenotipo DCL no amnésico posible, el subtipo con el menor riesgo y la menor tasa de conversión a demencia.

2. Los endofenotipos neuropsicológicos que mejor predijeron conversión a demencia para la muestra de DCL fueron la Orientación a la Realidad (tiempo, espacio y persona), Recuerdo Diferido de la Lista de Palabras de la WMS-III y los Relojes de Luria, independientemente de la presencia o ausencia de al menos un alelo ε4 del locus *APOE*.

3. En el estudio con biomarcadores un peor rendimiento en el *Composite* Cognitivo Recuerdo Diferido se encontró relacionado con una mayor carga de βA en el PET-PIB, un menor volumen hipocampal en la RM y un menor metabolismo en el PET-FDG. Estos resultados, obtuvieron replicación en la base de datos *Alzheimer's Disease Neuroimaging Initiative* (ADNI) para el PET-PIB, corroborando que el Recuerdo Diferido es el endofenotipo neuropsicológico óptimo para detectar la EA prodrómica, en comparación con los otros dominios cognitivos.

4. Todos los sujetos con el fenotipo DCL amnésico probable a nivel de almacenamiento y biomarcadores fueron positivos a nivel basal en, al menos, un biomarcador de neuroimagen y sólo 11 (55%) fueron positivos para los tres biomarcadores, mientras que en el endofenotipo neuropsicológico, todos obtuvieron un rendimiento alterado en el *Composite* Cognitivo Recuerdo Diferido en Memoria. Durante el seguimiento, la

mayoría (17/20, 85%) convirtió a demencia, principalmente (15/17, 88,2%) tipo EA y sólo 2 (11,8%) Demencia mixta (EA con enfermedad cerebrovascular asociada).

5. El meta-análisis sistemático realizado en cuatro series independientes y una amplia muestra de 3.226 sujetos con DCL, confirmó que, además del alelo ε4 del locus *APOE*, el locus Clusterina (*CLU*) es un factor genético independiente asociado con la fenoconversión de DCL a EA. Además, la puntuación combinada de genotipos (PGS1) que comprende nueve loci de riesgo de EA publicados con anterioridad al consorcio *International Genomics Alzheimer's Project* (IGAP) mostró un pequeño efecto sobre el riesgo en la fenoconversión de DCL a EA en los portadores de *APOE*-ε4.

6. El análisis de asociación entre endofenotipos neuropsicológicos y marcadores genéticos de la EA en la muestra de DCL ampliada hasta 1250 sujetos, confirmó, además del efecto de riesgo del locus *APOE*-ε4 asociado a la memoria episódica verbal de la Lista de Palabras de la WMS-III en el DCL, un efecto protector del locus *HS3ST1* (rs6448799) asociado al endofenotipo Dígitos Inversos de la WAIS-III y un efecto de riesgo del locus *AP2A2* (rs10751667) asociado a la Repetición verbal, en el fenotipo DCL amnésico probable.

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