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NEUROPSYCHOLOGICAL AND GENETIC TOOLS FOR THE DIAGNOSIS OF NEURODEGENERATIVE DISEASES PRESENTING WITH COGNITIVE AND/OR BEHAVIORAL IMPAIRMENT

Sara Rubio Guerra

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Neuropsychological and genetic tools for the diagnosis of neurodegenerative diseases presenting with cognitive and/or behavioral impairment

Doctoral Thesis

Sara Rubio Guerra



Barcelona, 2026

UAB Universitat Autònoma de Barcelona

DOCTORAL THESIS

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Barcelona, 2026

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A mi madre.

ACKNOWLEDGMENTS

A mis padres y a mi hermana, por ser mi raíz y apoyo incondicional.

A l'Oriol, per ser casa i per recordar-me cada dia quines són les coses més importants de la vida.

A mi abuelos, Vicenta, Paco, Antonia y Simón, y a mis tíos, Javier y Alfredo, por tanto amor y sacrificio.

A Víctor, Luisa y Juan Luis, por hacerme sentir siempre tan querida.

A Nuria, por tu temple y ejemplo de fortaleza.

A totes les persones que m'han acompanyat en aquest llarg camí d'aprenentatge i creixement personal:

Als professors del SA-FA i als de la universitat, per la vostra generosa i incansable dedicació a la formació de les noves generacions.

Al Xavier Navarro, per guiar-me al llarg dels anys.

Al servei de neurologia de Can Ruti, per ser família i per haver-me ensenyat les bases de la neurologia.

Al Josep, la Irina i la Gràcia, i als companys de medicina interna de l'Hospital de l'Esperit Sant, per la vostra acollida.

A les persones que integren la Unitat d'Alzheimer i altres trastorns cognitius de l'Hospital Clínic, per haver donat ales a la meva curiositat per la neurologia cognitiva i conductual.

A los *fellows*, y al resto de integrantes de la Unidad de Memoria y del servicio de neurología del Hospital de Sant Pau, ha sido un honor formar parte de esto.

A Tere, por tu cariño infinito.

A Manolo, por hacerme reír siempre.

A David y Fina, cada conversación con vosotros ha sido un aprendizaje para mí.

A la Lidia, la Sara, l'Anna i la Clara, per alimentar el meu interès per la genètica i posar tot el vostre coneixement al meu abast.

A mis directores de tesis, por darme la oportunidad de trabajar con vosotros y formarme en el mejor entorno posible. A l'Alberto, per confiar en mi i per ser referent d'integritat i excel·lència. A Ignacio, gracias por enseñarme que la pasión es una de las herramientas más valiosas de un investigador.

A los participantes y a los equipos asistenciales y de investigación que han hecho posibles estos estudios.

Finalment, vull donar les gràcies als meus pacients, de qui he après tantes coses, i a les seves famílies. És per vosaltres que aquesta tesi té sentit.

LIST OF ABBREVIATIONS

[^{18}F]FDG-PET	[^{18}F]fluorodeoxyglucose positron emission tomography
3R	three-repeat tau
4R	four-repeat tau
AD	Alzheimer's disease
ADAD	autosomal dominant Alzheimer's disease
ADNC	Alzheimer's disease neuropathologic change
ADRD	Alzheimer's disease and related neurodegenerative diseases
AFTD	Association for Frontotemporal Degeneration
AGD	argyrophilic grain disease
ALLFTD	ARTFL–LEFFTDS Longitudinal Frontotemporal Lobar Degeneration (where ARTFL = Advancing Research and Treatment in Frontotemporal Lobar Degeneration, LEFFTDS = Longitudinal Evaluation of Familial Frontotemporal Dementia Subjects)
ALS	amyotrophic lateral sclerosis
ALSFRS-R	Amyotrophic Lateral Sclerosis Functional Rating Scale–Revised
Amyloid PET	amyloid positron emission tomography
ANOVA	analysis of variance
APOE	apolipoprotein E
APP	amyloid precursor protein
ARIA	amyloid-related imaging abnormalities
ARIA-E	amyloid-related imaging abnormalities with edema
ARIA-H	amyloid-related imaging abnormalities with hemosiderin deposits (microhemorrhages and/or superficial siderosis)
ARTAG	aging-related tau astrogliopathy

ASO	antisense oligonucleotide
Aβ	amyloid β
Aβ−	amyloid β-negative
Aβ+	amyloid β-positive
Aβ40	amyloid β 1-40
Aβ42	amyloid β 1-42
BNT	Boston Naming Test
bvAD	behavioral variant of AD
bvFTD	behavioral variant of frontotemporal dementia
C9orf72	chromosome 9 open reading frame 72
CBS	corticobasal syndrome
cDNA	complementary DNA
CD	Clinical Dementia Rating
CDR-SB	Clinical Dementia Rating-Sum of Boxes
CERAD	Consortium to Establish a Registry for Alzheimer's Disease
CHMP	Committee for Medicinal Products for Human Use
CHMP2B	charged multivesicular body protein 2B
CI	confidence interval
CN	cognitively normal
COS	Cuestionario Oviedo de Sueño (Oviedo Sleep Questionnaire)
CRQ	Cognitive Reserve Questionnaire
CSF	cerebrospinal fluid
d	Cohen's d
DLB	dementia with Lewy bodies
DNA	deoxyribonucleic acid
DSAD	Down syndrome-associated Alzheimer's disease

ELISA	enzyme-linked immunosorbent assay
EMA	European Medicines Agency
ESCRT-III	endosomal sorting complex required for transport-III
FCSRT	Free and Cued Selective Reminding Test
FDA	Food and Drug Administration
FTD	frontotemporal dementia
FTD-ALS	frontotemporal dementia–amyotrophic lateral sclerosis spectrum disorder
FTD-rATL	frontotemporal dementia with right anterior temporal lobe predominance
FTLD	frontotemporal lobar degeneration
FTLD-FUS	frontotemporal lobar degeneration with fused in sarcoma inclusions
FTLD-S	frontotemporal lobar degeneration-related syndromes
FTLD-tau	frontotemporal lobar degeneration with tau inclusions
FTLD-TDP	frontotemporal lobar degeneration with TDP-43 inclusions
FTLD-UPS	frontotemporal lobar degeneration with ubiquitin proteasome system-positive inclusions
FUS	fused in sarcoma (gene *FUS*; protein FUS)
GAMLSS	generalized additive models for location, scale, and shape
GAMLSS distributions	
BB	beta-binomial distribution
BCPE	Box-Cox power exponential distribution
DBI	double-binomial distribution
NO	normal distribution

GAMLSS parameters	
μ	location parameter (e.g., mean)
σ	scale parameter (e.g., standard deviation)
ν	shape parameter (e.g., skewness)
τ	shape parameter (e.g., kurtosis)
GDS	Geriatric Depression Scale
GDS-FAST	Global Deterioration Scale and Functional Assessment Staging Tool
GENFI	Genetic Frontotemporal dementia Initiative
GFAP	glial fibrillary acidic protein
GRN	progranulin
HAD	Hospital Anxiety and Depression Scale
iADRS	Integrated Alzheimer's Disease Rating Scale
IDDD	Interview for Deterioration of Daily Living in Dementia
IPAQ	International Physical Activity Questionnaire
ISCED	International Standard Classification of Education
LATE-NC	limbic-predominant age-related TDP-43 encephalopathy neuropathological change
LBD	Lewy body disease
LME	linear mixed-effects
lvPPA	logopenic variant of primary progressive aphasia
MAPT	microtubule-associated protein tau
MBI	mild behavioral impairment
MCI	mild cognitive impairment
MFE-30	Memory Failures in Everyday
MIBG	[^{123}I]-metaiodobenzylguanidine myocardial scintigraphy
MMI	mild motor impairment

MMSE	Mini-Mental State Examination
MND	motor neuron disease
MoCA	Montreal Cognitive Assessment
MRI	magnetic resonance imaging
MTBR-tau243	microtubule-binding region tau containing residue 243
NfL	neurofilament light chain
nfvPPA	non-fluent variant of primary progressive aphasia
NGN	next-generation norms
NGS	next-generation sequencing
NMDA	N-methyl-D-aspartate
NPI	Neuropsychiatric Inventory
NSD	neuronal α-synuclein disease
NSS_A	Neuronorma Scaled Score-age adjusted
OPTN	optineurin
PCA	posterior cortical atrophy
PD	Parkinson's disease
PDD	Parkinson's disease dementia
PET	positron emission tomography
PPA	primary progressive aphasia
PSEN1	presenilin-1
PSEN2	presenilin-2
PSP-RS	progressive supranuclear palsy–Richardson syndrome
PSP-S	progressive supranuclear palsy syndrome
PSQ	Perceived Stress Questionnaire
p-tau	phosphorylated tau
p-tau181	phosphorylated tau at threonine 181
p-tau205	phosphorylated tau at threonine 205

p-tau217	phosphorylated tau at threonine 217
r	correlation coefficient
***r*²**	coefficient of determination
RBD	REM sleep behavior disorder
ROCF	Rey-Osterrieth Complex Figure
RP-PCR	repeat-primed polymerase chain reaction
rtvFTD	right temporal variant of frontotemporal dementia
sbvFTD	semantic behavioral variant of frontotemporal dementia
SD	standard deviation
SOD1	superoxide dismutase 1 (gene *SOD1*; protein SOD1)
***SOD1*-ALS**	amyotrophic lateral sclerosis due to pathogenic variants in the *SOD1* gene
SPECT	single photon emission computed tomography
SPIN	Sant Pau Initiative on Neurodegeneration
SQSTM1	sequestosome 1
Std. error	standard error
svPPA	semantic variant of primary progressive aphasia
T@M	Test de Alteración de Memoria (Memory Alteration Test)
TARDBP	transactive response DNA-binding protein
tau PET	tau positron emission tomography
TBK1	TANK-binding kinase 1
TDP-43	transactive response DNA-binding protein 43 kDa
TIA1	T-cell intracellular antigen 1
TMEM106B	transmembrane protein 106B
TMT-A/B	Trail Making Test Part A/Part B
TN	traditional norms
t-tau	total tau

UBQLN2	ubiquilin 2
UNESCO	United Nations Educational, Scientific and Cultural Organization
UPS	ubiquitin-proteasome system
VCP	valosin-containing protein
VOSP	Visual Object and Space Perception Battery
VUS	variant of uncertain significance
WAIS-III	Wechsler Adult Intelligence Scale–Third Edition
WAIS-IV	Wechsler Adult Intelligence Scale–Fourth Edition
WES	whole-exome sequencing
WGS	whole-genome sequencing
WMS–IV	Wechsler Memory Scale–Fourth Edition
α-syn SAA	α-synuclein seed amplification assay
η^2	eta-squared
φc	Cramér's V

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ABSTRACT

The therapeutic landscape of neurodegenerative diseases presenting with cognitive and/or behavioral impairment is undergoing a period of transformation. For the first time, two anti-amyloid β (Aβ) monoclonal antibodies and one gene-targeted treatment have been approved as disease-modifying therapies for Alzheimer's disease (AD) and the frontotemporal lobar degeneration (FTLD) spectrum, respectively.

Disease-modifying therapies must be initiated in the early stages of disease to maximize effectiveness. This thesis assesses the potential of neuropsychological and genetic tools to enhance the timeliness and accuracy of diagnosis of neurodegenerative diseases presenting with cognitive and/or behavioral impairment, thereby enabling early access to treatment.

First, we developed refined neuropsychological norms drawing both on evidence of the cognitive effects of preclinical Aβ pathology and the evolution of norming methods culminating in generalized additive models for location, scale, and shape (GAMLSS). Aβ-negative, GAMLSS-based normative data were derived for a comprehensive neuropsychological battery. Norms were adjusted for age, education, and sex, and were made available in an open-access, web-based calculator.

Then, we examined the added value of our norms in identifying the earliest stages of cognitive impairment. Compared with current standards, cognitive classifications under our norms demonstrated more robust associations with neuropsychological performance, AD biomarker positivity, and cognitive progression. Taken together, the results indicate that our norms enhance the detection of early cognitive impairment, especially that associated with AD. In particular, one in every five individuals currently classified as cognitively normal might be better diagnosed with mild cognitive impairment under our norms and thus potentially get earlier access to treatment. Our neuropsychological norms may inform biomarker use and candidate selection for therapeutic interventions.

We also explored the potential of genetic testing to improve the yield and timeliness of diagnosis of FTLD. We reported the cases of two monozygotic twins presenting in their 60s with a slowly progressive behavioral variant of frontotemporal dementia and a Lewy body-like dementia syndrome. Family history was notable for the aggregation of neuropsychiatric disorders of unknown etiology. Motivated by diagnostic uncertainty over follow-up, whole-exome sequencing of the proband identified a novel pathogenic variant in the *CHMP2B* gene, which was confirmed in both siblings, providing definitive evidence of underlying FTLD pathology. Our case report represents the first family with *CHMP2B*-associated FTLD in Spain, and adds to a growing body of literature supporting the offering of comprehensive genetic testing to all individuals with FTLD, regardless of family history.

Collectively, the studies presented in this thesis contribute to earlier and more accurate diagnosis of neurodegenerative diseases presenting with cognitive and/or behavioral impairment, with direct relevance to clinical practice and research.

RESUM

El panorama terapèutic de les malalties neurodegeneratives que es presenten amb deteriorament cognitiu i/o alteració conductual travessa un període de transformació. Per primer cop, dos anticossos monoclonals anti-amiloide β (Aβ) i un tractament dirigit contra un gen específic han estat aprovats com a teràpies modificadores de la malaltia d'Alzheimer (MA) i de l'espectre de la degeneració lobular frontotemporal (DLFT), respectivament.

Les teràpies modificadores han de ser iniciades a les etapes primerenques de la malaltia per maximitzar-ne l'efectivitat. Aquesta tesi avalua el potencial de les eines neuropsicològiques i genètiques per millorar la rapidesa i precisió del diagnòstic de les malalties neurodegeneratives que es presenten amb deteriorament cognitiu i/o alteració conductual, permetent l'accés precoç al tractament.

Primer, hem desenvolupat normes neuropsicològiques refinades basades tant en l'evidència sobre els efectes cognitius de la patologia Aβ preclínica com en l'evolució dels mètodes normatius, que ha culminat en els models additius generalitzats de posició, escala i forma (GAMLSS, per les sigles en anglès). A partir d'una mostra normativa Aβ-negativa i en base als models GAMLSS, hem computat valors normatius per a una bateria neuropsicològica extensa. Les normes han estat ajustades per edat, educació i sexe, i posades a disposició en una calculadora web d'accés obert.

A continuació, hem examinat el valorar afegit de les nostres normes en la identificació de les fases més precoces del deteriorament cognitiu. En comparació amb les normes vigents, la classificació cognitiva basada en les nostres normes mostra associacions més robustes amb el rendiment neuropsicològic, la positivitat dels biomarcadors de la MA i la progressió cognitiva. En conjunt, els resultats indiquen que les nostres normes milloren la detecció del deteriorament cognitiu incipient, particularment d'aquell associat amb la MA. En concret, un de cada cinc individus classificats actualment com a cognitivament normals seria diagnosticat de deteriorament cognitiu lleu

en base a les nostres normes i, per tant, potencialment obtindria un accés més precoç al tractament. Les nostres normes neuropsicològiques podrien informar l'ús dels biomarcadors i la selecció de candidats a intervencions terapèutiques.

També hem explorat el potencial de l'estudi genètic per aconseguir un diagnòstic més precoç i acurat de la DLFT. Hem reportat el cas de dos bessons monozigòtics, al voltant dels 60 anys d'edat, amb una forma lentament progressiva de la variant conductual de la demència frontotemporal i una síndrome semblant a la demència amb cossos de Lewy. De la història familiar destacava l'agregació de trastorns neuropsiquiàtrics d'etiologia desconeguda. Motivada per la incertesa diagnòstica durant el seguiment, la seqüenciació de l'exoma complet del proband va identificar una nova variant patogènica al gen *CHMP2B*, confirmada en els dos germans, aportant evidència definitiva de patologia DLFT subjacent. Aquests dos casos reportats representen la primera família a Espanya amb DLFT associada al gen *CHMP2B*, i contribueixen a un corpus creixent de literatura que recolza l'oferiment d'un estudi genètic ampli a tots els individus amb DLFT, independentment de la història familiar.

Col·lectivament, els estudis presentats en aquesta tesi contribueixen a un diagnòstic més precoç i acurat de les malalties neurodegeneratives que es presenten amb deteriorament cognitiu i/o alteració conductual, amb rellevància directa per a la pràctica clínica i la investigació.

1. INTRODUCTION

1. INTRODUCTION

1.1. The diagnostic framework of neurodegenerative diseases presenting with cognitive and/or behavioral impairment

In brief, the diagnosis of patients with cognitive and/or behavioral impairment due to suspected neurodegenerative disease follows a three-step approach: the first step involves assessing the overall cognitive-functional level of impairment; the second step is the delineation of the clinical syndrome; the third step is the pinpointing of the plausible underlying disease(s) causing the patient's symptoms and signs.(1,2)

Neurodegenerative diseases are classified according to the type of abnormal protein aggregates that accumulate in the central nervous system.(3)The most common neurodegenerative diseases presenting with cognitive and/or behavioral impairment are: Alzheimer's disease (AD, which accounts for 60-70% of neurodegenerative diseases that ultimately cause dementia(4) and is characterized by amyloid β [Aβ] and tau aggregates), Lewy body disease (LBD, with α-synuclein inclusions), and frontotemporal lobar degeneration (FTLD, most commonly with either transactive response DNA-binding protein 43 kDa [TDP-43] or tau inclusions). These pathological protein aggregates begin to accumulate focally in vulnerable, disease-specific regions, disrupting circuits involved in cognitive, behavioral, and motor functions, leading to distinct clinical features. The initially focal protein aggregates subsequently spread along specific networks, ultimately leading to global neurodegeneration. This protein propagation drives increasingly broad clinical impairment and progressive functional decline, culminating in dementia.(3,5,6)

Aging is the main risk factor for AD and related neurodegenerative diseases (ADRD), with prevalence increasing steadily with age.(7) In 2019, it was estimated that around 57 million people worldwide were living with dementia, an amount that is expected to dramatically increase to 153 million by 2050 due to population aging and growth. Sex is also a risk factor for dementia,

with females globally experiencing dementia 1.7 times more frequently than males.(8) Of note, females also disproportionately assume the years-long family caregiver role of people with dementia.(9) Both informal caregiving and direct health care impose mean annual costs ranging from 8,000€ to over 70,000€ per patient in different European regions (nearly 36,000€ in Southern Europe), which increase with disease severity.(10) With no cure available to date, ADRD represent the 7th leading cause of death globally (4th in high-income countries).(4)

Definitive diagnosis of ADRD is achieved through postmortem neuropathological examination (i.e., autopsy).(6) Nonetheless, since the 1980s, clinical diagnostic criteria have enabled antemortem prediction of the underlying pathology with varying degrees of certainty.(11–13) These criteria, which have since then undergone successive revisions, rely on the presence of core symptoms and signs characteristic of each disease. However, within the same disease, distinct patterns of protein aggregate deposition lead to variable clinical features and trajectories (i.e., phenotypic heterogeneity). Furthermore, there is phenotypic overlap both among neurodegenerative and between neurodegenerative and non-neurodegenerative diseases (e.g., vascular, psychiatric). Phenotypic heterogeneity and overlap thus pose significant challenges to clinical diagnosis.(14–17)

Since the 2000s, the field has shifted toward earlier, biologically confirmed diagnosis thanks to the advent of disease-specific biomarkers for some conditions.(18–22) Biomarkers are objective measures of underlying biological processes;(23) among these, biomarkers providing definitive evidence of the underlying proteinopathy are mainly classified into biofluid, imaging, and genetic modalities. These biomarkers have subsequently been integrated into updated diagnostic criteria. In essence, biofluid and imaging biomarkers now enable biological confirmation of AD,(24,25) while genetic biomarkers primarily provide a definitive diagnosis in certain cases of FTLD.(26,27)

Postmortem neuropathological and in vivo biomarker studies have expanded our understanding of neurodegenerative diseases, now conceptualized as

biological processes initiated by hallmark neuropathological changes years before symptom onset.(28) Under this conceptualization, three major stages are recognized in ADRD: the preclinical, prodromal, and dementia (or fully symptomatic) stages (**Figure 1**).

The *preclinical* stage is defined by biomarker (i.e., biofluid, imaging, genetic) or postmortem evidence of underlying pathology in the absence of objective clinical impairment.

The *prodromal* stage, which is the earliest symptomatic stage, is characterized by objective cognitive impairment, behavioral and/or motor change compared to previous performance, neither of which significantly interfere with daily functioning. The prodromal stage has been operationalized through the constructs of mild cognitive impairment (MCI) and mild behavioral impairment (MBI).(29–31) In recent years, research criteria have also been proposed for prodromal symptoms and signs in neurodegenerative diseases evolving to present with prominent motor deficits. In some instances, this prodromal syndrome has been given the analog term of mild motor impairment (MMI). (32,33)

The *dementia*, or *fully symptomatic* stage, is delineated by significant cognitive and/or behavioral impairment leading to loss of independent daily functioning, or otherwise interfering with social and/or occupational abilities despite intact independence, or by fully evolved motor changes.(24,25,34,35)

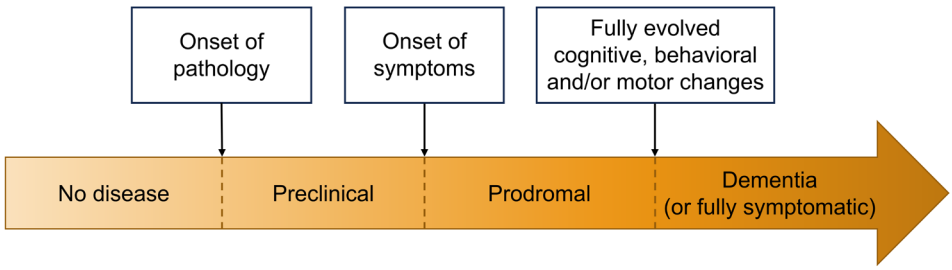


Figure 1. Clinical staging of neurodegenerative diseases presenting with cognitive, behavioral and/or motor changes. Adapted from and reconceptualized based on Benussi A, et al., *Alzheimers Dement,* 2022 (CC BY 4.0).(35)

Cognitive performance should ideally be established through a formal neuropsychological assessment, which is a validated method for evaluating brain function using cognitive test batteries, along with self- and informant-report questionnaires that assess behavioral and functional domains.(36) A comprehensive neuropsychological test battery should at least incorporate tests across the cognitive domains of memory, language, attention, executive functions, and visuospatial skills.(2,36,37)

The present thesis focuses on the diagnosis of neurodegenerative diseases presenting with cognitive and/or behavioral impairment. Within this field, it examines how neuropsychological and genetic tools can better contribute to diagnosis.

1.1.1. Alzheimer's disease

1.1.1.1. *Pathophysiology*

AD neuropathologic change (ADNC) is defined by the presence of extracellular Aβ neuritic plaques and intraneuronal phosphorylated tau (p-tau) neurofibrillary tangles.(38,39)

Building on these two primary pathological features, the prevailing pathophysiological model of AD – the amyloid cascade hypothesis – posits that extracellular accumulation of Aβ is the earliest pathological hallmark of the disease, and that this is followed by intracellular deposition of p-tau, synaptic dysfunction, and neuronal loss, culminating in clinical symptom onset.(40,41) The stage from initial Aβ deposition to symptom onset (i.e., the preclinical AD stage) may last as long as two decades.(42–44)

AD is most commonly a sporadic, late-onset disease; however, a minority of cases (<1%) are genetically determined.(45) Genetically determined AD includes early-onset cases (i.e., < 65 years) caused by pathogenic variants in the amyloid precursor protein (*APP*), presenilin-1 (*PSEN1*), and presenilin-2 (*PSEN2*) genes (autosomal dominant AD [ADAD]),(46) as well as Down syndrome-associated AD (DSAD, the most common genetic form of AD), in which ADNC develops nearly deterministically through *APP* triplication on

chromosome 21.(47) Beyond genetically determined AD, variants in other genes are associated with an increased risk of sporadic, late-onset AD. Among these, the apolipoprotein E (*APOE*) ***ε4*** allele represents the strongest genetic risk factor, with a gene dosage effect (i.e., risk increases from non-carriers to heterozygotes to homozygotes).(48) Recently, however, it has been proposed that *APOE* ***ε4*** homozygosity is actually a distinct form of genetically determined AD with near-complete lifetime penetrance.(49) Around 20-25% of the general population carry at least one *APOE* ***ε4*** allele, whereas 40-65% of individuals with an AD diagnosis are *APOE* ***ε4*** carriers.(50)

1.1.1.2. *Clinical presentation*

The most common clinical presentation of AD is the amnestic syndrome (**amnestic variant of AD**), characterized by progressive episodic memory deficits reflecting early medial temporal lobe pathology.(51,52) Atypical, non-amnestic presentations of AD reflect early neurodegeneration in other cortical regions.

Primary progressive aphasia (PPA) is a neurodegenerative clinical syndrome defined by language dysfunction being the most prominent feature from symptom onset. One of the subtypes of PPA, the **logopenic variant of PPA (lvPPA)**, has shown to be mostly related to underlying ADNC. Patients with the lvPPA present with single-word retrieval deficits and impaired repetition of long sentences.(27,53)

Posterior cortical atrophy (PCA) is a neurodegenerative clinical-radiological syndrome presenting with early visuospatial dysfunction, while other cognitive domains are initially relatively spared. AD is also the most common neuropathology underlying PCA, though it may also be caused by others, including LBD and FTLD.(54)

The **behavioral variant of AD** (**bvAD**, also known as frontal or executive variant) presents with early prominent behavioral and personality changes, as well as executive dysfunction.(55)

1.1.1.3. *Biomarkers*

Distinct patterns of atrophy and hypometabolism on magnetic resonance imaging (MRI) and ^{18}F-fluorodeoxyglucose positron emission tomography ([^{18}F]FDG-PET), as well as cerebrospinal fluid (CSF) or blood levels of neurofilament light chain (NfL) and glial fibrillary acidic protein (GFAP), reflect focal cortical accumulation of ADNC, secondary neurodegeneration, and astrocyte reactivity, respectively; however, these biomarkers are non-specific for AD.(24)

As of the 2024 revised criteria for diagnosis of AD, biomarkers allowing biological confirmation of the disease (i.e., core 1 biomarkers) provide evidence of the earliest detectable underlying ADNC (i.e., both Aβ neuritic plaques and tau neurofibrillary tangles). These include imaging and biofluid markers of Aβ and tau measuring insoluble and soluble aggregates, respectively: amyloid positron emission tomography (amyloid PET), the CSF hybrid ratios p-tau181/Aβ42, t-tau/Aβ42, and Aβ42/Aβ40, and newly introduced blood-based biomarkers (particularly the highly accurate p-tau at threonine 217 [p-tau217]).(24,25,56)

Later-changing biomarkers (i.e., core 2 biomarkers of ADNC) enable biological staging of neuropathologic burden across the AD continuum. Core 2 biomarkers include tau positron emission tomography (tau PET), CSF or plasma microtubule-binding region tau containing residue 243 (MTBR-tau243) and other p-tau forms (e.g., p-tau205), and CSF non-phosphorylated mid-region tau fragments.(24)

The 2024 revised criteria also delineate clinical stages for individuals in the AD pathophysiological continuum, as determined by the presence of biofluid, imaging or genetic biomarkers: stage 0 refers to asymptomatic individuals with genetically determined AD (i.e., ADAD, DSAD) but with otherwise negative biofluid and imaging AD biomarkers; stage 1 refers to asymptomatic individuals with biofluid or imaging biomarker evidence of ADNC (i.e., core 1 biomarkers); stage 2 is a transitional phase defined by subjective cognitive decline and/or recent-onset behavioral change (i.e., MBI) relative to previous level of functioning, or objective subtle cognitive decline on longitudinal cognitive assessments, but performing within normal range on cognitive tests

and not interfering with daily functioning; stage 3 is defined by impaired performance on objective cognitive tests without overt functional impact (i.e., MCI); stages 4-6 are defined by progressive cognitive-functional impairment (i.e., dementia).(24)

1.1.2. Lewy body disease

1.1.2.1. *Pathophysiology*

LBD neuropathology is characterized by intraneuronal inclusions of α-synuclein (Lewy bodies and Lewy neurites).(34,57)

In 70-80% of cases, LBD presents with other concomitant neuropathologies, most commonly AD.(58) AD copathology modifies the clinical presentation and disease course of LBD; hence, the pathological criteria for LBD involve assessing the probability that pathological findings – including ADNC– produce the clinical syndrome characteristic of the disease.(34,57)

Few highly penetrant pathogenic gene variants have been identified in LBD.(59)

1.1.2.2. *Clinical presentation*

From the initial 1996 consensus clinical diagnostic criteria, the term dementia with Lewy bodies (DLB) was adopted to designate the progressive dementia syndrome associated with cortical Lewy bodies.(12) Compared to AD patients, individuals with DLB commonly exhibit disproportionate attention/executive and visuospatial deficits. In some cases, visuospatial impairment may be the earliest and most prominent clinical feature, such that DLB is one of the differential diagnoses of PCA.(54) Individuals with DLB may also present with early or progressive deficits in language and memory. Memory deficits in DLB are usually milder than in AD, and are initially more pronounced for free recall, whereas they improve with cued recall.(60)

DLB is clinically heterogeneous; several non-cognitive subtypes have been described based on onset clinical features, namely: delirium-onset, neuropsychiatric-onset, motor-onset, and REM sleep behavior disorder (RBD)-onset.(61–63) Notwithstanding, per the 2017 clinical diagnostic criteria,

the diagnosis of DLB is not feasible until the dementia stage. More recently, research criteria have been proposed for the diagnosis of MCI with Lewy bodies; these newly proposed criteria closely parallel those of DLB by relying on core clinical features and indicative biomarkers.(62)

Core clinical features supporting the diagnosis of DLB are: fluctuating cognition and alertness, recurrent visual hallucinations, parkinsonism (bradykinesia, rigidity, and/or rest tremor), and RBD.(34)

1.1.2.3. *Biomarkers*

As per current diagnostic criteria, a diagnosis of probable DLB can be made on clinical grounds if any combination of at least two core clinical features is present. Otherwise, a diagnosis of probable DLB can be established if at least one core clinical feature is present and is accompanied by at least one indicative biomarker.(34)

Biomarkers indicative of DLB are: reduced dopamine transporter binding in the striatum, demonstrated by single photon emission computed tomography (SPECT) or PET imaging; reduced uptake in [^{123}I]-metaiodobenzylguanidine (MIBG) myocardial scintigraphy; and RBD confirmation in polysomnography. (34) Notably, none of the aforementioned biomarkers provides definitive evidence of underlying α-synuclein pathology.

At the beginning of this thesis, α-synuclein seed amplification assays (α-syn SAA) had shown high diagnostic performance as in vivo measures of LBD pathology in different biological tissues.(64,65) Albeit not yet readily available for wide routine clinical application, α-syn SAA promise to enable earlier, biologically confirmed diagnosis of LBD.

Historically conceived as independent clinical-pathological entities, biomarker advances are driving increasing recognition of DLB, Parkinson's disease (PD), Parkinson's disease dementia (PDD), and even isolated RBD, as a spectrum of clinical presentations of a single, biologically defined, neuronal α-synuclein disease (NSD). The NSD integrated clinical-biological staging is conceptually in line with that of AD.(66–68)

1.1.3. Frontotemporal lobar degeneration

1.1.3.1. *Pathophysiology*

FTLD is a heterogeneous neuropathological entity characterized by the relatively selective involvement of the frontal and temporal lobes of the brain. At a molecular level, FTLD can be divided into four main categories based on the biochemical composition of the protein aggregates that accumulate in neurons and glia: tau (FTLD-tau) and TDP-43 (FTLD-TDP) are the most common underlying protein inclusions, each equally accounting for about 45% of cases; the remaining cases are associated with underlying fused in sarcoma (FUS) inclusions (FTLD-FUS, 9%) or ubiquitin proteasome system (UPS)-positive inclusions that are negative for tau, TDP-43, and FUS (FTLD-UPS, 1%) (**Figure 2**).(69,70)

Both FTLD-tau and FTLD-TDP are further classified into subtypes according to the composition, morphology, and distribution of protein inclusions. FTLD-tau molecular subtypes are defined according to the predominant tau isoform composition resulting from alternative splicing of the *MAPT* gene (encoding microtubule-associated protein tau): three-repeat (3R) tau-predominant, four-repeat (4R) tau-predominant, or mixed 3R/4R tau pathology.(69) In turn, FTLD-TDP pathological classification, historically encompassing types A to D,(69) was recently expanded by the proposal of a fifth subtype (type E).(71)

FTLD has a strong genetic component, with about 20-25% of cases carrying an autosomal dominant variant in a known FTLD-causing gene. By far, the most common genetic cause of FTLD is a hexanucleotide (GGGGCC) repeat expansion in the *C9orf72* gene (encoding chromosome 9 open reading frame 72).(20,72) The other two leading FTLD-causing genes are *GRN* (encoding progranulin)(21) and *MAPT*.(22) Together, variants in *C9orf72*, *GRN*, and *MAPT* account for the vast majority of genetically confirmed FTLD cases, while the remainder are associated with variants in other genes, including *VCP, TARDBP, TBK1, SQSTM1, TIA1, UBQLN2, OPTN, FUS*, and *CHMP2B* (for full gene names, see the list of abbreviations; **Figure 2**). Genetic forms of FTLD are frequently associated with a younger age of onset.(73,74)

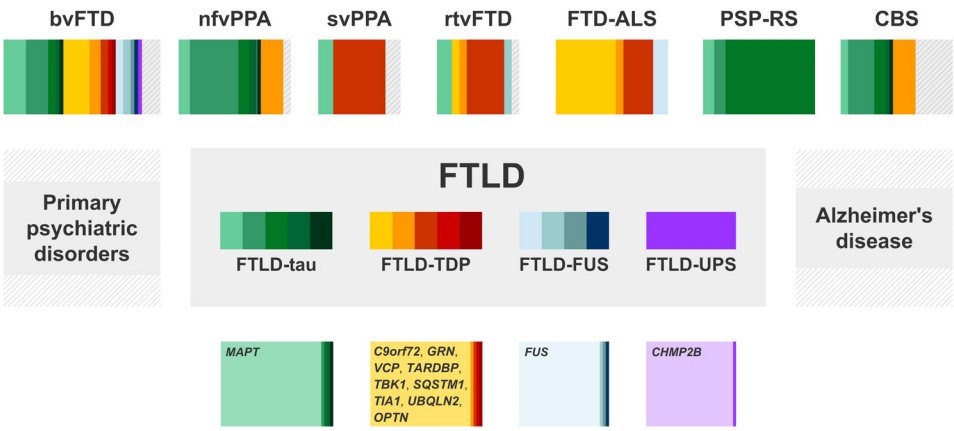


Figure 2. Schematic representation of the clinical, pathological, and genetic heterogeneity of FTLD. The top row shows the major FTLD-S (i.e., bvFTD, nfvPPA, svPPA, rtvFTD, FTD-ALS, PSP-RS, CBS) colored according to the approximate frequencies of underlying etiologies, which are displayed in the middle row. FTLD pathology is classified into distinct molecular subtypes (i.e., FTLD-tau, FTLD-TDP, FTLD-FUS, and FTLD-UPS). Primary psychiatric disorders and AD account for a proportion of patients with FTLD-S. The bottom row presents the main gene variants associated with each FTLD subtype. Inspired by the conceptual framework presented in *Dickerson BC & Hodges JR*, 2016.(75)

Abbreviations: bvFTD, behavioral variant of frontotemporal dementia; CBS, corticobasal syndrome; FTD-ALS, frontotemporal dementia–amyotrophic lateral sclerosis spectrum disorder; FTLD, frontotemporal lobar degeneration; FTLD-FUS, frontotemporal lobar degeneration with fused in sarcoma inclusions; FTLD-S, frontotemporal lobar degeneration-related syndromes; FTLD-tau, frontotemporal lobar degeneration with tau inclusions; FTLD-TDP, frontotemporal lobar degeneration with TDP-43 inclusions; FTLD-UPS, frontotemporal lobar degeneration with ubiquitin proteasome system-positive inclusions; nfvPPA, non-fluent variant of primary progressive aphasia; PSP-RS, progressive supranuclear palsy–Richardson syndrome; rtvFTD, right temporal variant of frontotemporal dementia; svPPA, semantic variant of primary progressive aphasia; for full gene names, see the list of abbreviations.

1.1.3.2. *Clinical presentation*

FTLD is also clinically heterogeneous; it may initially present with behavioral and personality changes, speech and language deficits, and pyramidal or extrapyramidal motor impairment.

Frontotemporal dementia (FTD) is the clinical term encompassing three major syndromes classically associated with underlying FTLD pathology: the **behavioral variant of frontotemporal dementia (bvFTD),** the **non-fluent variant of PPA (nfvPPA)**, and the **semantic variant of PPA** (**svPPA**, also known as semantic dementia).(76)

FTLD most commonly presents with bvFTD, which is characterized by early personality change, including behavioral disinhibition, apathy or inertia, loss of empathy, stereotyped or compulsive behavior, hyperorality, and increased consumption of toxic substances (e.g., alcohol or cigarettes). Neuropsychological impairments in bvFTD patients usually include deficits in executive functions and social cognition, while episodic memory and visuospatial skills are relatively preserved.(26,77) The other FTD clinical syndromes are two subtypes of PPA: the nfvPPA and the svPPA.

Patients with the nfvPPA may exhibit agrammatism in language production and/or slow, effortful speech with speech sound errors; they might also present with impaired comprehension of syntactically complex sentences, with sparing of single-word comprehension and object knowledge.(27) Whether this phenotypic heterogeneity reflects distinct pathological entities has generated considerable debate, but evidence now supports they exist along a spectrum with shared pathological correlates.(78,79)

On the other hand, patients with the svPPA exhibit fluent speech with deficits in confrontation naming and word comprehension; they can also present with impaired object knowledge, and surface dyslexia or dysgraphia (i.e., difficulty reading and writing words in which there is an unusual relationship between spelling and pronunciation). Other language-dependent cognitive tasks, such as verbal episodic memory, might well also be impaired.(27)

As will be outlined in the following section, the diagnosis of svPPA is strongly supported by the neuroimaging finding of a canonical pattern of left-predominant anterior temporal lobe atrophy. Recently, the clinical phenotype associated with counterpart right-predominant anterior temporal lobe atrophy has been described. This clinical-radiological syndrome has been named **right temporal variant of frontotemporal dementia** (**rtvFTD**), also referred to as semantic behavioral variant of frontotemporal dementia (sbvFTD), or frontotemporal dementia with right anterior temporal lobe predominance (FTD-rATL). Patients with the rtvFTD present with prominent behavioral changes and loss of non-verbal semantic knowledge, such as impaired recognition of faces and emotions. On neuropsychological examination, they commonly exhibit prominent episodic memory deficits and language impairment (e.g., word-finding difficulties).(80–83)

In recent times, it has been recognized that FTLD can also manifest in the form of pyramidal or extrapyramidal motor dysfunction.

Amyotrophic lateral sclerosis (ALS), the most common form of motor neuron disease (MND), is characterized by the involvement of both upper motor neurons (i.e., pyramidal tracts) and lower motor neurons, and may present with hyperreflexia, spasticity, clonus, bulbar and/or limb weakness, muscle atrophy, and fasciculations.(84,85) While ALS has been traditionally understood as a purely motor syndrome, a substantial body of research has revealed that it exhibits marked clinical, pathological, and genetic overlap with FTD. Over the past years, this overlap has given rise to the concept of an FTD-ALS spectrum disorder (**Figure 3**).(74,86)

Clinical overlap. Besides motor deficits, it has been observed that around 50% of individuals diagnosed with ALS exhibit cognitive and/or behavioral impairment. Cognitive deficits in ALS patients most frequently involve executive functions, language, and social cognition, whereas apathy is the most common behavioral change.(87) Conversely, it has been shown that up to 40% of FTD cases manifest motor neuron symptoms or signs.(88) Approximately 15% from each end of the spectrum (i.e., patients with either motor or cognitive-behavioral onset) meet the criteria for both ALS and FTD (most commonly, bvFTD).(74,86–88) Co-ocurrence of ALS and FTD confers a worse prognosis over pure ALS or FTD presentations, with a median survival of 2.8 years.(89)

Pathological overlap. The link between ALS and FTD was confirmed by the 2006 identification of the underlying TDP-43 protein inclusions,(90) which are the pathological hallmark of 97% of ALS cases and nearly half of FTD cases (**Figure 3A**).(70)

Genetic overlap. For decades, pedigrees demonstrating FTD, ALS and both FTD-ALS have been documented. The causative locus was successively narrowed down, leading to the discovery of the shared GGGGCC repeat expansion in *C9orf72* in 2011.(20,72) While the *C9orf72* repeat expansion is the most common genetic cause across the FTD-ALS spectrum, additional genes contribute to the overlap between FTD and ALS (e.g., *TBK1, SQSTM1, VCP, TARDBP, OPTN, UBQLN2*). Additionally, pathogenic variants in non-overlapping genes cause isolated FTD (e.g., *GRN*, *MAPT*) or ALS (e.g., *SOD1*, superoxide dismutase 1) (**Figure 3B**).(74,91,92)

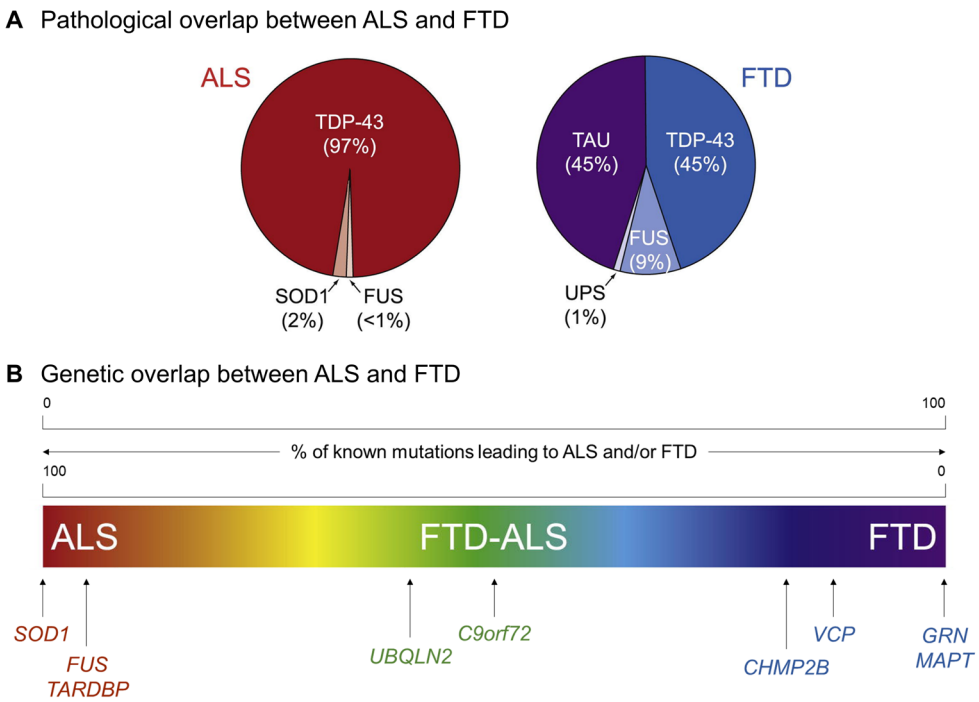


Figure 3. Clinical, pathological, and genetic overlap between ALS and FTD. (A) Proportion (%) of pathological protein aggregates underlying ALS and FTD across major molecular subtypes, with overlapping TDP-43 and FUS inclusions. (B) Proportion (%) of known mutations causing ALS and/or FTD. FTD-ALS genetic overlap is represented in the middle of the bar. Adapted from Ling S, et al., *Neuron*, 2013(70) (Copyright © 2013 Elsevier), with permission from Elsevier. Minimal adaptations include updating gene symbols to standard forms and formatting for clarity.

Abbreviations: ALS, amyotrophic lateral sclerosis; FTD, frontotemporal dementia; FTD-ALS, frontotemporal dementia–amyotrophic lateral sclerosis spectrum disorder; for full protein and gene names, see the list of abbreviations.

Two extrapyramidal motor syndromes have been associated with FTLD: **progressive supranuclear palsy syndrome (PSP-S)** and **corticobasal syndrome (CBS).**

PSP-S encompasses a heterogeneous group of clinical entities characterized by variable degrees of ocular motor dysfunction, postural instability, parkinsonism, and cognitive-behavioral impairments. Core neuropsychological deficits in

PSP-S include executive dysfunction and impairment of speech and language. (93)

CBS is characterized by a combination of motor and higher cortical features, typically with right- or left-predominant asymmetry. Core motor features include limb rigidity or akinesia, limb dystonia, and limb myoclonus, while higher cortical features comprise orofacial or limb apraxia, cortical sensory deficits, and alien limb phenomena. On cognitive examination, patients with CBS may present with executive, speech and language, and visuospatial impairments (in some cases, visuospatial deficits can fulfill criteria for PCA). (54,94)

All these clinical phenotypes have been associated with underlying FTLD pathology, and are collectively termed FTLD-related syndromes (FTLD-S) (**Figure 2**).

Despite recent updates in clinical diagnostic criteria, strict categorization into separate FTLD-S proves difficult given high phenotypic heterogeneity and overlap. Instead, there is a growing consensus that FTLD-S do not represent discrete entities, but rather overlapping multidimensional clinical manifestations of FTLD pathology.(95)

1.1.3.3. *Biomarkers*

Neuroimaging studies, including MRI and [^{18}F]FDG-PET, aid in identifying the specific regional patterns of atrophy and hypometabolism associated with each FTLD-S. In bvFTD patients, neuroimaging may demonstrate bilateral involvement of the frontal and anterior temporal brain regions.(26,96) Patients with nfvPPA and svPPA typically show left-predominant atrophy and hypometabolism of the posterior fronto-insular and anterior temporal regions, respectively.(27) By contrast, the recently described rtvFTD is characterized by right-predominant focal degeneration of the anterior temporal lobe.(80) Patients with ALS may exhibit early bilateral degeneration of the motor cortex and corticospinal tract.(97) Finally, in PSP-S, neuroimaging may reveal predominant midbrain atrophy or hypometabolism, while patients with CBS

may show asymmetric atrophy and hypometabolism of the perirolandic cortex.(73,98)

These neuroimaging patterns are reflective of the early epicenters of neurodegeneration, and their anatomical and functional brain network connections, which enhances the certainty of the clinical diagnosis.(99) However, as shown above, FTLD is pathologically, genetically, and clinically heterogeneous. With few exceptions, each FTLD-S may have multiple underlying FTLD molecular classes, and each FTLD pathological subtype can result in several phenotypes in different individuals. These weak clinical-pathological correlations render the antemortem prediction of the specific pathology difficult (**Figure 2**).(75)

Three salient exceptions exist for the otherwise poor clinical-pathological correlations in FTLD. First, up to 97% of ALS cases show FTLD-TDP pathology, mostly of type B.(70,75,90,100) Second, in around 80% of svPPA patients, FTLD-TDP type C is the underlying neuropathology.(73,101) Lastly, progressive supranuclear palsy–Richardson syndrome (PSP-RS), the late-stage converging phenotype of the PSP-S spectrum, is strongly associated with underlying 4R-tau FTLD in up to 90% of cases (**Figure 2**).(73,102)

With these exceptions, antemortem definitive diagnosis of underlying FTLD pathology and its specific molecular subtype can only be reached through genetic testing, in which each known FTLD-causing gene is associated with a specific proteinopathy: the *C9orf72* repeat expansion, along with pathogenic gene variants in *GRN, VCP, TARDBP, TBK1, SQSTM1, TIA1, UBQLN2*, and *OPTN* are associated with specific subtypes of FTLD-TDP pathology; *MAPT* variants are associated with underlying FTLD-tau pathology; *FUS* variants lead to FTLD-FUS; finally, *CHMP2B* variants predict underlying FTLD-UPS (**Figure 2**). Thus, in the context of a phenotype consistent with an FTLD-S, a positive genetic test makes the diagnosis definitive. In contrast, biomarkers providing definitive evidence of FTLD pathology in non-genetic forms of FTLD remain notably absent.(73)

Nevertheless, a recent multicenter study on the quantification of TDP-43 and tau in plasma extracellular vesicles has shown excellent discrimination accuracy in a cohort with ALS, PSP-S and bvFTD patients, including some neuropathologically-confirmed cases. This study opens the door for the emergence of an in vivo biomarker of FTLD pathology in non-genetic cases. (103)

Phenotypic overlap of FTLD-S with other diseases –mainly AD and primary psychiatric disorders– further challenges the etiological diagnosis (**Figure 2**).(75) AD-specific imaging and biofluid biomarkers aid in the differential diagnosis between AD and FTLD. In turn, CSF or blood levels of NfL, a non-specific marker of neuroaxonal damage, can be useful to differentiate between a primary psychiatric disorder and FTLD. In the context of an FTLD-S, the combination of negative AD biomarkers and high CSF or blood NfL concentrations increases diagnostic certainty of underlying FTLD pathology. (76,104,105)

1.2. The transformative therapeutic landscape of neurodegenerative diseases presenting with cognitive and/or behavioral impairment

Psychological distress, social stigma, nihilism emerging from the absence of disease-modifying therapies, shortage of specialists, and lack or limited access to high-performing diagnostic tools are among the obstacles to timely diagnosis of neurodegenerative diseases presenting with cognitive and/or behavioral impairment.(106)

However, a diagnosis made while the individual is still functionally independent can help the patient adjust to their symptoms, plan for the future, and make vital decisions (i.e., financial, legal, care-related, etc.). An early diagnosis can also aid families in making sense of otherwise seemingly random symptoms, and caregiver education and support can help them cope better with the psychological burden associated with the caregiver role.(75,106,107)

A timely diagnosis enables the implementation of available non-pharmacological and pharmacological interventions. In particular, a diagnosis

in the prodromal stage allows greater time margin for the modification of risk factors that may reduce incident dementia, such as correction of hearing and visual loss, reduction of social isolation, cessation of smoking and excessive alcohol consumption, control of hypertension, high LDL cholesterol and diabetes, reduction of obesity, and promotion of physical activity.(108,109) Non-pharmacological treatments include environmental modifications, psychosocial interventions, cognitive rehabilitation, speech and language therapy, and use of compensatory tools to enhance communication, physical and occupational therapy, use of thickeners, walkers, and/or wheelchairs.(73,75,110,111) Such approaches are applicable across all causes of cognitive-behavioral impairment. On the other hand, symptomatic medications for cognitive impairment comprise cholinesterase inhibitors (i.e., donepezil, rivastigmine, and galantamine) and the N-methyl-D-aspartate (NMDA) receptor antagonist memantine.(112–115) Cholinesterase inhibitors counteract the cholinergic deficiency in AD and DLB, whereas memantine is primarily used for AD.(111,116) Extrapyramidal motor symptoms may respond to levodopa in some patients with DLB, CBS, and PSP-S. In addition, antidepressants can ameliorate some psychological and behavioral symptoms, such as depressive features, anxiety, irritability, disinhibition, compulsive behaviors, and hyperphagia. Antipsychotic medications may also be helpful to treat hallucinations, delusions, agitation, aggression, and nightly unrest.(75,110,117) Critically, these pharmacological and non-pharmacological interventions improve symptom management, but do not target underlying disease mechanisms; thus, they do not delay or reverse disease progression. By contrast, riluzole, which stands out as the first and most widely approved medication for ALS worldwide, yields a modest increase in survival of 2-3 months by reducing glutamate-mediated excitotoxicity.(118,119)

After decades of fruitless research for disease-modifying treatments, when this thesis was started in 2022, emerging AD anti-Aβ antibodies and FTLD gene-targeted therapies promised to transform the therapeutic landscape of these diseases.

In June 2021, aducanumab –a monoclonal antibody targeting Aβ aggregates in early AD– received accelerated approval by the US Food and Drug Administration (FDA) based on evidence for Aβ reduction in amyloid PET as a surrogate marker of clinical efficacy in two phase III clinical trials (EMERGE and ENGAGE). For its part, the European Medicines Agency (EMA) did not follow in FDA's footsteps, but rejected aducanumab due to concerns about the clinical benefits and risks of Aβ clearance.(120–122) Although not without controversies, aducanumab marked a milestone by becoming the first approved disease-modifying therapy for AD.(123)

In FTLD, the development of potential disease-modifying therapies has been driven by genetic forms of the disease, which allow reliable antemortem prediction of the underlying FTLD pathology.(73) Hence, at the outset of this thesis, most clinical trials in FTLD evaluated gene-targeted treatments, while only a few focused on non-genetic forms with strong clinical-pathological correlations (e.g., PSP-RS, which robustly predicts underlying FTLD-tau pathology). Gene-targeted trials at that time included potential therapies for the *C9orf72* repeat expansion, and for pathogenic variants in *GRN, MAPT*, and *SOD1*.(76,124) However, these were mostly early-phase trials and, at the start of this thesis, no gene-targeted therapies had been approved for FTLD.

The long-awaited disease-modifying therapies for ADRD are most likely to be effective in the early stages of the disease. Thus, although the empowerment of patients and carers and the implementation of lifestyle and symptomatic interventions justify the importance of early detection in its own right, the emergence of potential disease-modifying therapies now requires unprecedented diagnostic refinement.

1.3. Motivation for the study

There are two main reasons justifying the present thesis. First, reliance on neuropsychological evaluation to inform the indication and interpretation of AD biomarkers underscores the need for accurate neuropsychological norms

to enable early detection and intervention. Second, concerning FTLD, the identification of a pathogenic gene variant provides a definitive diagnosis of the underlying pathology and may determine a patient's eligibility for gene-targeted treatments, while also having important familial and research implications.

The arrival of anti-Aβ antibodies highlights the importance of early, biologically confirmed AD diagnosis, as these treatments show the greatest efficacy at earlier disease stages. However, in clinical settings, it is recommended that AD biomarkers not be used in asymptomatic individuals.(24,25) This recommendation is based on two reasons: the absence of preventive treatments, and the limited accuracy of biomarkers for predicting lifetime cognitive decline in asymptomatic individuals.(125,126) As outlined previously, the distinction between the preclinical (i.e., asymptomatic) and prodromal (i.e., early symptomatic) stages depends on the objective demonstration of cognitive impairment in a neuropsychological assessment.

In cognitively impaired individuals, biomarkers aid in solving the differential diagnosis associated with phenotypic heterogeneity and symptom overlap. However, pure neuropathological substrates of cognitive-behavioral impairment are rare; in most cases, several pathologies contribute to varying clinical features and disease trajectories (e.g., vascular disease, LBD, FTLD, limbic-predominant age-related TDP-43 encephalopathy neuropathological change [LATE-NC], argyrophilic grain disease [AGD], aging-related tau astrogliopathy [ARTAG]).(2,125,127–129) Given the absence of biomarkers for most non-AD neurodegenerative diseases, and that biomarkers alone are not yet adequate to establish whether biological evidence of pathology accounts for the patient's symptoms, it is recommended that biomarker findings be interpreted in the context of a detailed clinical assessment, including a comprehensive neuropsychological evaluation that characterizes the profile of cognitive deficits.(2,24,28,25,126)

In the past two decades, two key milestones toward refined neuropsychological norms have occurred in parallel: the neuropsychological characterization of the preclinical AD stage, and the evolution of the statistical models used to derive normative data.

Following the advent of AD biomarkers, an important body of literature has emerged on the cognitive effects of preclinical AD.(130–136) This evidence has raised concern about how the unintended inclusion of individuals with preclinical AD in normative samples might impact norm-derived clinical judgment, and has prompted the development of AD-negative neuropsychological norms.(136–141)

Parallel to biomarker advances, statistical models used for norming have undergone substantial evolution, culminating in the development of generalized additive models for location, scale, and shape (GAMLSS),(142) with yet scarce but promising applications to neuropsychological norming.(143,144)

Despite the advances in knowledge about the cognitive effects of preclinical AD and progress made in the area of norming approaches, to date, these refinements have not been jointly translated into practice.

The second reason for this thesis concerns the identification of all genetic cases of FTLD, with broad implications for patients and their families, as well as for research. Although pathogenic variants in known FTLD-causing genes are most frequently identified in familial forms of the disease, these variants have also been found in apparently sporadic cases (i.e., with no known family history). On the other hand, a considerable proportion of dominantly inherited familial cases lack an identified genetic cause.(145)

However, despite technological advances that increasingly make genetic testing feasible, there is a lack of clinical practice guidelines on genetic testing for patients with FTLD-S, and practices differ substantially across physicians and centers.(146,147)

1.4. Thesis aim

This thesis aims to elucidate how neuropsychological and genetic tools can enhance the timeliness and accuracy of diagnosis of neurodegenerative diseases presenting with cognitive and/or behavioral impairment, ultimately improving treatment access.

Of note, throughout this thesis, the terms "timely" and "early" diagnosis will be used interchangeably to refer to a diagnosis made at a time when the patient first consults for concerns about changes in cognition and/or behavior relative to their baseline level of functioning. Importantly, these terms do not refer to a diagnosis made at the earliest possible (i.e., preclinical) phase.

1.5. Structure of the thesis

This doctoral thesis comprises eight further sections.

In **Section 2**, I elaborate on the foundational knowledge for this work and dissect the research gaps that justify the present thesis.

In **Sections 3** and **4**, I set the specific hypotheses and objectives of the study based on accumulated knowledge.

The objectives of this thesis have materialized in the publication of three articles, for all of which I am first author, and which are presented in **Section 5**. Supplementary materials are provided in **Annexes 1** and **2** of this thesis.

Section 5.1. describes the development of novel neuropsychological norms jointly through the selection of an AD-negative normative sample and the implementation of the GAMLSS framework.

Section 5.2. examines the performance of the newly developed neuropsychological norms in detecting early cognitive impairment relative to current norms.

Section 5.3. explores, through a case study, the potential of contemporary techniques for the universal and comprehensive genetic screening in individuals with FTLD-S.

In **Section 6**, I provide a global summary of the results.

Lastly, **Sections 7**, **8**, and **9** contain the thesis's integrated discussion, as well as the conclusions and future directions.

2. RATIONALE OF THE STUDY

2. RATIONALE OF THE STUDY

2.1. Neuropsychological assessment

2.1.1. Diagnostic contributions of neuropsychological assessment

As outlined in the Introduction, the first step in the diagnosis of ADRD involves assessing the degree of cognitive-functional impairment (i.e., cognitively normal, MCI, or dementia): this staging is based on clinical criteria. Here, particularly challenging is the distinction between normal cognition and MCI, depending on impaired performance in one or more cognitive domains that does not otherwise interfere with daily functioning.(1,2,37,60) There is no single agreed-upon definition of cognitive impairment: several definitions exist depending on the selected cutoff for test impairment and the number of impaired tests required for diagnosis.(148–150) Compared to bedside cognitive screening tools, comprehensive neuropsychological assessment enhances early detection of cognitive impairment.(36,37) Moreover, the profile of neuropsychological deficits permits topographic mapping of neurodegeneration,(151) ultimately informing etiological differential diagnosis and biomarker interpretation.(36,37,60,125) Therefore, despite the remarkable progress in biomarkers, neuropsychological assessment adds value to the three diagnostic steps for ADRD, thus contributing to more timely and accurate diagnosis.(37)

With the advent of disease-modifying therapies, there is a growing demand for enhanced neuropsychological assessment tools to aid in the detection and characterization of the earliest objective cognitive deficits.

2.1.2. Neuropsychological norms

An individual's performance on a certain neuropsychological test is interpreted in relation to the test performance of the reference population. This comparison is achieved using neuropsychological norms, which enable

transformation of the individual's raw test score into a norm-referenced score derived from a sample of the reference population (i.e., normative sample). Upon transformation of the raw score into a norm-referenced score, neuropsychological test impairment is typically defined as performance that is 1, 1.5, or 2 standard deviations (SD) below the normative mean.(36)

Currently, the most widely used neuropsychological norms in Spain date back to 2009 (the Neuronorma Project developed by Peña-Casanova J et al.). The Neuronorma Project provided the first Spanish comprehensive battery of neuropsychological normative data, laying the foundations for the identification and characterization of cognitive impairment in our country. (152)

2.1.2.1. *Impact of preclinical neuropathologies on cognitive performance*

A normative sample is the benchmark by which an individual's performance is compared; thus, the composition of the normative sample may affect norm-derived clinical judgment. Specifically, the inclusion of impaired individuals in normative groups has been shown to lower the normative mean test scores while increasing variability. This results in smaller differences when comparing the normative group with the test's target population, thereby decreasing overall classification accuracy.(153)

Cognitive decline in late life is at least partially driven by the presence of one or more, age-related brain neuropathologies –including AD, LBD, and FTLD–, which begin to accumulate in cognitively unimpaired (preclinical) individuals years or decades before symptom onset. Importantly, even in the absence of overt symptoms, preclinical neuropathologies affect cognitive performance. (154)

The impact of preclinical neuropathologies on normative estimates of cognitive aging has long concerned the field.(155) However, only with the recent advent of accurate biomarkers has their effect on cognitive performance been adequately measured. At the outset of this doctoral thesis, the most robust evidence on the effects of preclinical neuropathologies centered on AD, primarily embodied in a 2017 meta-analysis by Baker JE et al.(130)

Brain Aβ accumulation, which is regarded as the earliest necessary event in AD, has consistently been shown to impair cognitive performance in asymptomatic individuals. In particular, individuals with Aβ pathology show deficits in episodic memory, executive and visuospatial functions, and global cognition, albeit with small effect sizes.(130–136) Although small, the high prevalence of Aβ pathology among cognitively unimpaired older adults – increasing from 10% to 23% to 44% at ages 50, 70, and 90 years, respectively (156)– may yield profound implications for estimates of normal cognitive aging if individuals with preclinical AD are inadvertently included in normative samples.

Building on this concern, several earlier works have derived neuropsychological norms from AD biomarker-negative normative samples.(136–141) Collectively, a number of these studies conclude that AD-negative neuropsychological norms improve diagnostic sensitivity for cognitive impairment, but that this may be at the expense of specificity.(136,138–141) To date, no consensus has been reached on the appropriateness of biomarker-negative norms (157) and, most frequently, current neuropsychological norms don't account for biomarker status.

2.1.2.2. *Demographic influences on cognitive performance*

It has long been recognized that neuropsychological performance is affected by demographic variables such as age and education; instead, only in recent years has sex been considered a core normative variable.(158,159) Accordingly, current international expert consensus recommends that neuropsychological norms account for age, education, and biological sex (which is typically determined by the presence of either an XX or an XY chromosome complement(160)) to allow comparison of an individual's performance with that of their reference population (i.e., demographically matched cognitively normal individuals).(157)

Age has been associated with a decline in performance mainly in fluid abilities (i.e., those requiring processing speed, abstract reasoning, and quick and flexible decision making, such as measures of executive function), while crystallized abilities (i.e., cumulative knowledge and skills learned through

education, cultural exposure, and experience, such as vocabulary) remain relatively well preserved.(36,161,162) The extent to which the age effect on cognitive performance is explained by preclinical neuropathologies remains to be fully understood.(138)

Educational level (e.g., years of education) –among other related proxies of cognitive reserve, such as occupational performance and engagement in cognitively demanding activities– has a positive association with neuropsychological performance in all cognitive domains across the lifespan. The association between education and cognitive performance is slightly stronger for crystallized abilities, including language-related tests, than for fluid abilities.(162,163)

In addition to age and education, in the last decade, sex differences in neuropsychological performance have increasingly been recognized as important for normative modeling and norm-derived clinical diagnosis. (159,164) The cumulative body of evidence generally supports the existence of domain-specific sex advantages, with females outperforming males in verbal episodic memory tasks, and males outperforming females in spatial episodic memory measures and visuospatial abilities.(159,165,166) Regarding language and attention/executive function, no overall sex advantage has been reported, but rather task-specific sex differences.(165–169) Sex differences in cognitive abilities may result from distinct biological (e.g., hormonal), but also environmental (e.g., gender stereotypes) influences from the first stages of life,(164,165) and remain largely stable throughout the lifespan.(159,165,166)

Increased awareness of cognitive sex differences has prompted the concern that diagnosis of cognitive impairment may be overlooked if neuropsychological norms are not sex-adjusted.(159) Because differences in cognitive performance between females and males are domain-specific, and sometimes task-specific, norms that are unadjusted for sex could lead to sex-specific diagnostic errors when these domains, or tasks, are a hallmark of the patient's disease. This is the case for verbal episodic memory deficits in AD: the female advantage in verbal episodic memory, which is maintained into the early stages of the disease,(170–172) has been shown to result in delayed diagnosis of amnestic

MCI in females when sex-unadjusted norms are used. By contrast, diagnostic accuracy is improved by the use of sex-specific neuropsychological norms. (173–175)

2.1.2.3. *Norming approach*

Norm-referenced scores are derived from the raw scores obtained by the normative sample at the test(s) in question. The normative sample must be representative of the reference population and large enough to ensure that the resulting norms are both generalizable and precise.(176) Representativeness of the normative sample relates to its composition, which should ideally mirror the proportions of demographics and other relevant characteristics (e.g., cultural background) of the reference population, thus enabling generalizability of the norms. While these traits should be proportionally represented in the normative sample, it should be noted that not all necessarily influence performance on each specific test.(176)

There are currently two major norming approaches for neuropsychological tests: traditional norming and regression-based norming. The key difference between them lies in the method by which each approach accounts for relevant variables, and its resulting impact on sample size efficiency.

2.1.2.3.1. Traditional norming

Traditional norming (otherwise called stratified norming) divides the normative sample into subgroups, one for each combination of relevant variables (e.g., age, education, and sex), which are selected based on statistical significance, effect size, or their use in stratification of the normative sample to ensure representativeness. Then, separate norm-referenced scores (i.e., descriptive mean and SD) are derived for each demographically-homogeneous subgroup (e.g., 60-69 years old, compulsory education, male). This stratification intuitively leads to a reduced sample size within each normative subgroup, undermining the precision of norm estimates. However, beyond reduced sample size, even more problematic is that continuous variables (e.g., age, education) must be categorized into arbitrary intervals (e.g., age ranges, educational levels), biasing interpretation of test scores: arbitrary categorization of continuous variables

assumes that the performance is homogeneous within defined intervals, while it may change abruptly between consecutive intervals (e.g., 60-69 years, 70-79 years) –thus, an age difference of merely a few days could lead to a different interpretation of an individual's cognitive performance. The intuitive solution of defining more, narrower intervals (e.g., 60-65 years) would only exacerbate the reduced sample size problem, leading to less precise norms.(176,177)

2.1.2.3.2. Regression-based norming

In 1985, linear regression models were proposed as a solution to avoid categorizing continuous variables, hence the designation regression-based norming (a type of so-called continuous norming).(178) In regression-based norming, all relevant variables (including continuous ones, such as age) are incorporated as covariates in statistical models to predict the normative sample's mean raw scores, while the other distribution parameters (such as SD) are assumed constant. Derived norm-referenced scores quantify an individual's raw score deviation from their demographically adjusted expected performance, expressed in SD units (e.g., z-scores). By allowing the inclusion of continuous covariates, regression-based norming makes more efficient use of the sample size (i.e., utilizing the entire normative sample for norm estimation) and enables more realistic test score interpretation, ultimately enhancing norm precision.(176,177)

Notwithstanding the advantages of linear regression models, their applicability is conditioned on stringent assumptions, including normality, linearity, and homoscedasticity of the data.(176,177) A normal distribution is a bell-shaped symmetric distribution in which the response variable (i.e., raw test scores of the normative population) clusters around the mean and becomes less frequent as it moves away from it. In turn, linearity refers to a relationship in which a change in the predictor variable (e.g., age) produces a proportional change in the response variable. Finally, homoscedasticity refers to a constant variance across the range of values of the response variable. However, most neuropsychological tests do not meet these assumptions. For instance, many neuropsychological measures exhibit floor or ceiling effects (in which raw test scores cluster at the test's minimum or maximum values, respectively,

altering both the distribution of the data and its variance), or display nonlinear relationships between predictors and response variables.(36,177,179) For this reason, since the introduction of linear regression models, regression-based norming has undergone substantial evolution in the search for more flexible models. This evolution culminated in the 2005 introduction of generalized additive models for location, scale, and shape (GAMLSS).(142)

GAMLSS is a flexible framework that allows modeling response variables (i.e., raw test scores of the normative sample) which may follow more than 100 distinct distributions. GAMLSS further supports modified versions of the base distributions – including their truncated and censored versions, which account for the test's theoretical raw score ranges as well as floor/ceiling effects. Thus, GAMLSS overcomes the assumption of normal distribution inherent in linear regression. Additionally, it incorporates the possibility of modeling non-linear relationships between predictors and the response variable, thus also overcoming the linearity assumption of linear regression models. Finally, unlike linear regression, which estimates two distribution parameters (i.e., the mean raw score modeled as a function of covariates, and SD which is constant across observations), GAMLSS allows independent modeling of up to four distribution parameters, each modeled as an additive function of the selected predictor variables: μ (location parameter, e.g., mean); σ (scale parameter, e.g., SD); ν and τ (shape parameters, e.g., skewness and kurtosis, respectively). Crucially, each GAMLSS distribution parameter (μ, σ, ν, τ) may be best explained by a different combination of predictor variables, each of which may have a linear or non-linear relationship with the respective parameter.(177)

In regression-based norming in general, and GAMLSS in particular, stepwise regression is the most widely used strategy to select the appropriate predictor variables to be included in the final model. Stepwise selection involves adding (forward selection) and/or removing (backward selection) predictors in sequential steps, based on statistical criteria, until the final model is reached. (176,180) In GAMLSS, the most used stepwise approach is Strategy A, which applies forward selection sequentially for each distribution parameter (μ, σ, ν, τ), followed by backward selection in reversed order using the statistical criterion of Generalized Akaike Information Criterion.(180)

Previous studies have applied GAMLSS to derive normative data for a limited number of cognitive tests: the Mini-Mental State Examination (MMSE), the Montreal Cognitive Assessment (MoCA), the Color Trails Test, and the MemTrax task.(143,144)

2.1.2.4. *Current neuropsychological norms*

The current Spanish norms (Neuronorma Project, 2009) were originally derived from a multicenter normative sample of 356 individuals aged over 49 years.(152) Later, in 2012, additional normative studies were published with a complementary normative sample of 179 young adults aged between 18 and 49 years.(181) Key inclusion criteria for the normative groups (Neuronorma Project, 2009, 2012) were cognitive normality and independent daily functioning, as defined by a score above or equal to 24 on the age- and education-adjusted Spanish version of the MMSE (range 0-32)(182,183), a score below 37 on the Spanish version of the Interview for Deterioration of Daily Living in Dementia (IDDD, range 33-99)(184,185), and overall clinical judgement. Of note, while no criteria were applied based on the participants' biomarker status, this option could probably not have been considered because biomarkers were not yet widely available.

The original Neuronorma Project (2009) is half way between traditional and regression-based norming. Based on the traditional norming approach, the first step in the Neuronorma method involved stratifying the normative sample into age intervals (here, an overlapping age interval strategy —by which each normative individual contributed to more than one age interval— was implemented to maximize the sample size in each age subgroup), on which separate linear regression models were fitted next. However, prior to fitting linear regression models, an intermediate step was conducted to ensure that the normality assumption was met: raw scores in each age range were assigned percentile scores, which were subsequently transformed into age-adjusted scaled scores (NSS_A, Neuronorma Scaled Score-age adjusted). Ultimately, separate linear regression models were fitted on normalized NSS_A, with education and sex incorporated as covariates where deemed relevant.

The Neuronorma Project (2009) examined the effects of age, education, and sex on cognitive performance. To this end, for each test they analyzed linear associations between raw scores and each demographic variable using *r* correlation coefficients (range -1 to +1, representing the direction and strength of the linear association, with 0 indicating no linear relationship) and r^2 coefficients of determination (range 0 to 1, indicating the proportion of variance in one variable explained by the other). The inclusion of education and/or sex in the linear regression model was a priori determined by an r^2 0.04 criterion (i.e., the predictor was included when it explained more than 4% of the raw score variance). NSS_A were then further corrected for education and/or sex when these explained more than 4% of the NSS_A variance. Education and sex adjustments were made through regression coefficients (β), with education standardized to a reference of 12 years of education. Remarkably, while education was found to have a significant effect on nearly all tests normed in the Neuronorma Project (2009), significant sex effects were only observed in the semantic fluency tasks of naming "fruits and vegetables" and "kitchen tools" (sex adjustments were made to account for a female advantage in both tasks).(186) Therefore, the Neuronorma Project (2009) accounted for age and education nearly universally, and sex only exceptionally.

Overall, through initial age interval stratification, the original Neuronorma Project (2009) is primarily grounded in traditional norming, with its previously described intrinsic limitations, yet it secondarily integrates regression-based methods for final model building via linear regression. Nevertheless, the intermediate step of transforming raw scores into scaled scores to achieve a normal distribution often fails to detect low scores, compared with fitting linear regression models that use raw scores as the response variable.(187) Another limitation of the Neuronorma method is its reliance on a univariate linear association effect size metric (r^2) to select demographic predictors for final model building, which ignores covariate intercorrelations and non-linear relationships with the test score.(176,188) Likewise, the use of linear regression to adjust for education (and in a few cases for sex) assumes linearity and homoscedasticity of the data, not accounting for possible non-linear relationships or non-constant variance.

Whilst the Neuronorma Project represented a major advance in 2009, it is time to review neuropsychological normative data in light of new tools and improved methods that have emerged in recent years.

2.2. Genetic testing

2.2.1. Relevance of genetic diagnosis in FTLD

In the absence of other biomarkers specific for FTLD pathology, the identification of a genetic cause enables definitive diagnosis of FTLD, and may determine a patient's eligibility for gene-targeted therapies. Moreover, should a pathogenic variant be identified in a symptomatic individual, their family members can learn their own risk of developing the disease and thus make informed decisions regarding genetic testing and reproductive planning. Finally, through involvement in research, both symptomatic and presymptomatic carriers of a pathogenic gene variant can contribute to scientific understanding of the disease and development of novel gene-targeted therapies.(76,189) Thus, identifying genetic forms of FTLD is crucial for both patients and their family members, as well as FTLD research.

However, at the beginning of this doctoral thesis, consensus guidelines on genetic testing for individuals with FTLD-S remain elusive. In this context, diagnostic testing practices vary significantly across practitioners and centers.(147,189)

The most common approach is to reserve genetic testing for cases with a positive family history of the disease.(75,147,190,191) A familial disease is generally understood as one that occurs in several cases within the same family, as opposed to sporadic disease cases, which refer to individuals without a known family history of the disease.(145) Genetic testing in familial cases most commonly involves screening for the *C9orf72* repeat expansion (the most prevalent genetic cause of FTLD); in some cases, additional analysis of *GRN* and *MAPT* is performed.(76)

2.2.2. Diagnostic genetic testing in FTLD: challenges and opportunities

2.2.2.1. *Unreliable family history*

Among individuals with an FTLD-S, approximately 25-45% are considered familial disease cases, while the remaining 55-75% are regarded as sporadic.(75,145,192) Pathogenic variants in known FTLD-causing genes account for 50-70% of familial disease cases. Remarkably, up to 20% of apparently sporadic cases also harbor a pathogenic gene variant, most frequently the *C9orf72* repeat expansion (**Figure 4**).(74,145,192–195)

The lack of apparent family history in some genetic cases of FTLD may be attributable to multiple factors. First, there is no consensus on the definition of familial disease. Inconsistent classification of familial versus sporadic disease may arise from the different inclusion of first-degree-only to second- or third-degree family members; the level of certainty of FTLD diagnosis –ranging from a clinical diagnosis to genetically or pathologically confirmed FTLD–; and the inclusion or exclusion of neurodegenerative and psychiatric diseases, as genetic forms of FTLD not uncommonly mimic the clinical presentations of other diseases (e.g., AD, LBD, PD, Huntington's disease, bipolar disease, schizophrenia).(74,145,192,196,197) Second, a specific FTLD genetic variant may present with distinct clinical phenotypes across different family members; therefore, familial disease may go undetected if some phenotypes are overlooked during family history taking (e.g., failure to inquire about familial antecedents of ALS during the evaluation of a patient with bvFTD).(145,192) Third, ascertainment of familial FTLD may be hindered by underdiagnosis of affected family members, as well as early death or lack of knowledge about previous generations.(145,197,198) Fourth, small family size, and incomplete or age-dependent gene penetrance (i.e., the proportion of carriers of a risk genotype that manifest the related phenotype) may mask an autosomal dominant pattern of disease inheritance as seemingly sporadic.(74,192,199) Fifth, although infrequent, *de novo* mutations in several genes have been described (e.g., *C9orf72, FUS, SOD1*).(73,200) Lastly, some genes (e.g., *TMEM106B*) may play a role as disease modifiers in individuals carrying a pathogenic gene variant (e.g., *C9orf72*, *GRN*).(145,201)

Despite the lack of clinical practice guidelines, before this thesis, an increasing recognition of the limitations of family history taking, and the relatively high incidence reported for the *C9orf72* repeat expansion in apparently sporadic cases, had generated a growing awareness of the potential advantages of offering testing for the *C9orf72* repeat expansion to any individual with an FTLD-S, irrespective of family history. Meanwhile, testing for other known FTLD-causing genes remained mostly limited to cases with a compelling family history.(92,189)

2.2.2.2. *Unexplained family history*

Despite increasing awareness that pathogenic gene variants may be identified in a proportion of individuals with apparently sporadic FTLD-S, the diagnostic yield of genetic testing is substantially higher in individuals with a strong family history than in seemingly sporadic cases (50-70% vs 10-20%). Notably, the other 30-50% of individuals with definitive family history remain genetically unexplained (**Figure 4**).(74,75,145)

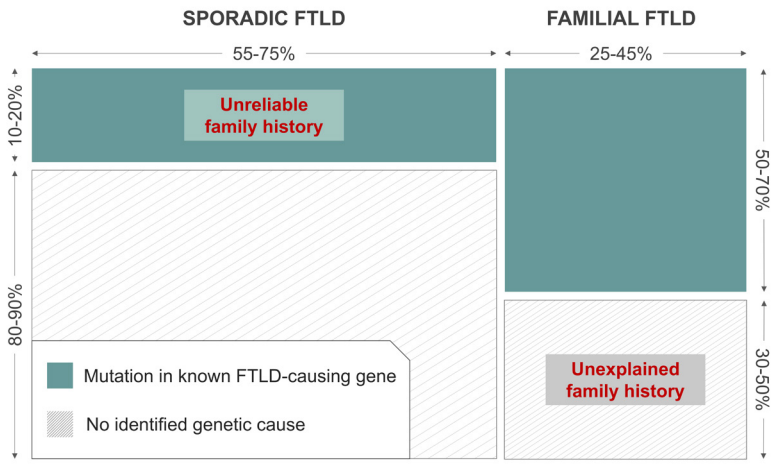


Figure 4. The horizontal (column-wise) classification reflects the proportion (%) of sporadic and familial FTLD cases. The vertical classification (row-wise) reflects the proportion (%) of individuals in each group with a mutation in some known FTLD-causing gene. Notably, 10-20% of apparently sporadic cases harbor a pathogenic variant in a gene associated with FTLD; conversely, 30-50% of familial cases remain genetically unexplained.

Abbreviations: FTLD, frontotemporal lobar degeneration.

Unexplained heritability in familial FTLD may be due to several reasons. One possible reason is that the genetic study may have insufficient coverage of less frequent, or newly discovered genes.(147) Yet, another potential explanation is that, in some of these families, FTLD may be caused by still undiscovered genes.(145)

2.2.2.3. *Advances in genetic testing techniques*

Until a few years ago, genetic testing had to be conducted on a single-gene basis. This required time, and genes studied had to be prioritized according to the index of suspicion. Single-gene testing techniques include Sanger sequencing, repeat-primed polymerase chain reaction (RP-PCR), and Southern blot.(146)

Over the last few years, methods of gene testing have advanced significantly. Previous single-gene testing techniques have been largely replaced by so-called next-generation sequencing (NGS) methods, which allow simultaneous analysis of multiple genes. NGS comprises multi-gene panel testing, whole-exome sequencing (WES) and whole-genome sequencing (WGS).(146)

Gene panel testing is currently the most widely used NGS method. It involves the simultaneous study of multiple genes, previously selected based on their similar related clinical phenotypes. WES and WGS enable analysis of the entire exome (coding regions, representing 1% of the genome) and genome (all DNA – both protein-coding and non-coding– in an organism or cell), respectively. The high diagnostic capacity of WES and WGS is counterbalanced by their increased likelihood of incidental findings. For this reason, and because of the high cost of processing large data volumes, the most common approach following WES and WGS is to restrict the analysis of the exome/genome to a set of genes related to the phenotypic presentation, with the advantage that WES/WGS may be reviewed in light of future gene discoveries, and may themselves lead to the discovery of new genes associated with the disease.(146)

Despite the advantages offered by the different NGS techniques, these fail to reliably detect repeat expansions, such as the *C9orf72* GGGGCC hexanucleotide expansion. The *C9orf72* repeat expansion can be detected using RP-PCR and

Southern blot.(189) On the other hand, Sanger sequencing remains the gold standard in confirming the presence of a known familial mutation in at risk relatives.(146)

While no evidence-based guidelines on genetic testing for FTLD existed at the beginning of this thesis, recent advances in NGS increasingly render universal and comprehensive genetic testing both efficient and economically feasible.

3. HYPOTHESES

3. HYPOTHESES

1) The combination of Aβ− normative sampling and GAMLSS modeling, along with improved selection of predictor variables, may yield refined neuropsychological normative data, resulting in enhanced accuracy in identifying the earliest stages of cognitive impairment compared with current standards.
2) Low-threshold comprehensive genetic testing of individuals with FTLD-S may enhance both the yield and timeliness of FTLD diagnosis, with multiple implications for patients, their families, and the research field.

4. OBJECTIVES

4. OBJECTIVES

Main objective:

To investigate how neuropsychological and genetic tools can enhance the timeliness and accuracy of diagnosis of neurodegenerative diseases presenting with cognitive and/or behavioral impairment.

Specific objectives:

1) To develop Aβ−, GAMLSS-based normative data for a comprehensive neuropsychological battery, adjusted for sex as well as age and education as appropriate.
2) To compare the performance of refined neuropsychological norms in detecting cognitive impairment with that of current normative data.
3) To illustrate the clinical and research relevance of comprehensive genetic testing upon diagnosis, or suspicion, of some FTLD-S.

5. COMPENDIUM OF PUBLICATIONS

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5.1. Article 1: Development of amyloid-negative neuropsychological norms using GAMLSS

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Received: 12 March 2025 | Revised: 10 September 2025 | Accepted: 31 October 2025

DOI: 10.1002/dad2.70224

Alzheimer's & Dementia
Diagnosis, Assessment & Disease Monitoring

RESEARCH ARTICLE

Development of amyloid-negative neuropsychological norms using GAMLSS

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Funding information
Ajuntament de Barcelona; Alzheimer's Association; Alzheimer's Society; Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas; Department of Economic Promotion of the Provincial Government of Gipuzkoa; Department of Health of the Basque

Abstract

INTRODUCTION: Recent research has suggested increased sensitivity of Alzheimer's disease (AD)-negative neuropsychological norms; concurrently, generalized additive models for location, scale, and shape (GAMLSS) have emerged as a promising alternative to traditional norming approaches. Here, we developed amyloid β-negative (Aβ–) next-generation norms (NGN) for a comprehensive neuropsychological battery using GAMLSS.

METHODS: We included $N = 987$ cognitively normal (CN) individuals from a Spanish multicenter study with extensive neuropsychological data and cerebrospinal fluid AD biomarker assessment. NGN were developed using GAMLSS based on the performance of $n = 774$ Aβ– CN individuals aged 30–90 years.

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Government; European Union; Fondo Europeo de Desarrollo Regional; Fundació Catalana Síndrome de Down; Fundació 'la Caixa'; Fundació La Marató de TV3; Fundación Tatiana Pérez de Guzmán el Bueno; Fundació Víctor Grífols i Lucas; Generalitat de Catalunya; Global Brain Health Institute; Instituto de Salud Carlos III; Jérôme Lejeune Foundation; National Institutes of Health

RESULTS: Age-, education-, and sex-adjusted *z*-scores were obtained for 14 measures covering the main cognitive domains (memory, language, attention/executive, and visuospatial functions). A user-friendly calculator for the *z*-scores was made available in an open-access ShinyApp to facilitate their application.

DISCUSSION: NGN may improve the detection of objective cognitive impairment in clinical and research settings.

KEYWORDS

Alzheimer's disease, amyloid β, GAMLSS, neuropsychological norms, normative data

Highlights

- Brain amyloid β (Aβ) is associated with poorer performance in cognitively normal individuals.
- We provide GAMLSS-based Aβ-negative norms for 14 neuropsychological measures.
- Age, education, and often sex significantly influence cognitive performance.
- An online calculator for the demographically adjusted *z*-scores is freely available.

1 | BACKGROUND

Alzheimer's disease (AD) and related neurodegenerative diseases are now conceptualized as biological continuums initiated by asymptomatic neuropathological changes.[1–3] In AD, the revised diagnostic and staging criteria delineate a progression through several stages: (1) asymptomatic individuals with biomarker evidence of AD-related neuropathological change; (2) a transitional phase characterized by subjective or objective decline in cognitive or neurobehavioral functioning compared to the individual's previous level of performance, but still within normal limits on objective cognitive tests; (3) abnormal cognitive performance while maintaining functional independence; and (4 to 6) progressive cognitive and functional impairment.[1] Timely and accurate detection of cognitive impairment is essential for biomarker use, therapeutic interventions, and selecting participants for research studies. Aligning with AD stage 3, mild cognitive impairment (MCI) of any cause represents the intermediary stage between normal cognition and dementia. While impaired independent functioning distinguishes dementia from earlier stages, the distinction between normal cognition and MCI relies on the precise identification of cognitive impairment, underscoring the need for robust normative data on cognitive performance.[4–7]

Developing accurate neuropsychological norms presents several challenges, a fundamental one being the composition of the normative sample. Brain amyloid β (Aβ) accumulation has been associated with cognitive impairment from the preclinical stages of AD.[8–11] Notably, previous studies have demonstrated increased sensitivity of AD-negative neuropsychological norms[11–15]; however, there is no consensus regarding their utility due to concerns of reduced specificity, and traditional norms do not account for biomarker status.

A second major challenge concerns the norming approach. Traditional norming categorizes continuous predictors (e.g., age) into arbitrary intervals. This leads to imprecise estimates due to a loss of statistical power and the assumption that score changes occur abruptly between (e.g., age) intervals. Furthermore, traditional norming methods typically model only the mean response, potentially leading to unrealistic results. In contrast, regression-based norming using generalized additive models for location, scale, and shape (GAMLSS) offers an efficient and flexible alternative by including continuous covariates and modeling all distribution parameters beyond the mean.[16,17] GAMLSS have been successfully implemented in earlier works to calculate normative values for the Mini-Mental State Examination (MMSE), the Montreal Cognitive Assessment (MoCA), and the Color Trails Test in Ireland,[18] and for the MemTrax in China.[19]

Although existing studies have independently developed AD-negative norms and applied GAMLSS within the field of neuropsychology, there remains a gap in research that integrates both these advances.

This multicenter study aims to develop Aβ-negative (Aβ–) next-generation norms (NGN) for a comprehensive neuropsychological battery using the GAMLSS framework. By addressing current norm development challenges, this study seeks to enhance the precision of cognitive classification, advancing the field's capabilities for early diagnosis and treatment of AD and related disorders.

RESEARCH IN CONTEXT

1. **Systematic review**: The authors searched PubMed for norming approaches for neuropsychological tests. Alzheimer's disease (AD)-negative neuropsychological norms may enhance sensitivity for detecting objective cognitive impairment, and generalized additive models for location, scale, and shape (GAMLSS) have emerged as a promising alternative to traditional norming approaches. To our knowledge, there are no published studies on the development of GAMLSS-based AD-negative norms.
2. **Interpretation**: In this large multicenter study, we applied GAMLSS to develop amyloid β-negative norms for 14 neuropsychological measures spanning memory, language, attention/executive, and visuospatial functions. Norms were adjusted for age, education, and sex. To facilitate their application, we developed an open-access ShinyApp that retrieves adjusted *z*-scores from raw test scores and covariates.
3. **Future directions**: Further studies could include additional neuropsychological tests, broader biomarker panels, and more diverse normative populations.

2 | METHODS

2.1 | Participants and design

Data were prospectively collected as part of the longitudinal multicenter SIGNAL study, which harmonized core neuropsychological procedures across several Spanish centers.[20] For the present study, we retrospectively selected $N = 987$ cognitively normal (CN) individuals who were recruited between March 2006 and July 2022. Participants were recruited from both outpatient memory clinics and community-based volunteer populations. The inclusion criteria were as follows: (1) normal cognition (see Table S1 for a summary of the tests and criteria used to classify CN individuals at each center), (2) age ≥ 30 years (due to an underrepresentation of younger individuals), and baseline availability of both (3) a neuropsychological evaluation and (4) cerebrospinal fluid (CSF) AD biomarker assessment, including Aβ status. The exclusion criterion was a history of neurological, psychiatric, or systemic conditions that might affect cognitive performance (Figure S1). This study adhered to the 1964 Declaration of Helsinki and was approved by the ethics committees of all participating centers. Written informed consent was obtained from all participants before enrollment.

To derive NGN, we defined a normative dataset comprising all A$\beta-$ CN individuals ($n = 774$).

2.2 | Materials and procedure

2.2.1 | Neuropsychological evaluation

All participants completed a baseline neuropsychological examination according to their center's protocol (Table S1). The assessment incorporated the MMSE (range 0–30, with lower scores reflecting greater impairment) as a measure of general cognitive functioning.[21] For this study, we selected those neuropsychological tests common to all participating centers for which there were published Spanish normative data (the Neuronorma Project[22,23]). The selected tests included at least two measures from each cognitive domain across visuospatial skills, memory, attention/executive function, and language (Table 1).

This neuropsychological battery conforms to international standards for the assessment of individuals with suspected cognitive impairment.[35]

2.2.2 | CSF biomarkers of AD

All participants underwent a baseline lumbar puncture to analyze CSF AD biomarkers, of which Aβ 1-42 (Aβ42) was the most widely available Aβ biomarker across centers. Individuals were classified as either Aβ-positive (A$\beta+$) or A$\beta-$ based on each center's cutoff for Aβ42 (Supplementary Methods).

2.3 | Statistical analyses

Statistical analyses were conducted using R, version 4.2.3.[36] Two-group comparisons of continuous variables with equal variances were conducted using *t*-tests; for unequal variances, we applied Welch's *t*-test. Between-group differences in categorical variables were examined using chi-squared tests. Statistical significance for all tests was set at p value < 0.05 ($\alpha = 0.05$). Standardized effect size statistics were calculated to quantify the magnitudes of between-group differences: we reported Cohen's d (d) for *t*-tests and Cramér's V (φc) for chi-squared tests. The magnitudes of the differences were interpreted as small, medium, or large based on generally accepted thresholds.[37] To account for demographic influences when comparing neuropsychological performance between groups, we calculated *w*-scores (standardized residuals) using a fully adjusted regression approach. For each neuropsychological measure, a multiple linear regression model was fitted in the A$\beta-$ CN reference group. Based on clinical knowledge, age, education, and sex were included as covariates in all models, irrespective of their univariate or multivariate significance. The predicted value for each participant was subtracted from their observed raw score and divided by the model's residual standard deviation, yielding a *w*-score that represents individual deviation from demographically adjusted expected performance.

TABLE 1 Neuropsychological measures.

Cognitive domain	Neuropsychological test	Raw score
Visuospatial	VOSP number location[24,25]	Total number of correct responses (0–10)
	ROCF copy[26,27]	Total number of correct elements (0–36)
Memory	FCSRT total free recall[26,27]	Sum of 16-item free recall scores across three trials (0–48)
	FCSRT total recall[26,27]	Sum of 16-item free + cued recall scores across three trials (0–48)
	FCSRT delayed free recall[26,27]	16-item delayed free recall (0–16)
	FCSRT delayed total recall[26,27]	16-item delayed free + cued recall (0–16)
	ROCF delayed recall[26,27]	Total number of correct elements (0–36)
Attention/executive	TMT-A[28,29]	Completion time measured in seconds (s; floor score, 150 s)
	TMT-B[28,29]	Completion time measured in seconds (s; floor score, 300 s)
	Digit Span Forward[28,29]	Length of the longest correctly repeated series (0–9)
	Digit Span Backward[28,29]	Length of the longest correctly repeated series (0–8)
	Phonetic fluency[30,31]	Total number of words beginning with P generated in 1 minute (*n*)
Language	Semantic fluency[30,31]	Total number of animal names generated in 1 minute (*n*)
	BNT[32–34]	Percentage (%) of correct spontaneous + semantic cued responses from either the 60- or the 15-item version upon availability; if both were available, the 60-item version was preferred.

Note: Neuropsychological measures within each cognitive domain, and their raw scores. Missing neuropsychological data in the normative dataset accounted for 8.6% of observations.

Abbreviations: BNT, Boston Naming Test; FCSRT, Free and Cued Selective Reminding Test; ROCF, Rey-Osterrieth Complex Figure; TMT-A/B, Trail Making Test Part A/Part B; VOSP, Visual Object and Space Perception Battery.

2.4 | Computation of NGN

We used GAMLSS to derive NGN based on the performance of n = 774 Aβ− CN participants. Neuropsychological measures were modeled using relevant distributions—including their truncated and censored versions, as required to account for floor and ceiling effects. Each candidate distribution allowed modeling for up to four parameters that determined its location (μ), scale (σ), and shape (ν, τ). These parameters were defined as additive functions of the predictor variables age, education, and sex. Age and years of education were analyzed as continuous variables, while sex was included as a dichotomous variable. We used smoothing terms (i.e., polynomials and P-splines) to account for non-linear relationships between continuous predictors and the outcome variable. Next, we used a forward and backward stepwise approach, based on the Generalized Akaike Information Criterion, to select the optimal combination of predictors for each distribution parameter.[38] Candidate models for each neuropsychological measure were then compared using statistical criteria (Akaike Information Criterion) and visual diagnostic methods (worm plots) to get the optimal model, from which norm-referenced z-scores were obtained. Analyses were performed using the GAMLSS R package[16,39] as described by Timmerman ME, et al.[17] We developed an interactive open-access ShinyApp[40] to enable users to obtain age-, education-, and sex-adjusted z-scores for every neuropsychological measure.

3 | RESULTS

3.1 | Demographics and baseline characteristics

Baseline characteristics of CN participants, stratified by Aβ status, are shown in Table 2. Aβ− CN individuals (i.e., the normative dataset) were younger and had more years of education than Aβ+ CN individuals. After adjusting for demographic variables, Aβ+ CN individuals showed a small but significant cognitive impairment in verbal memory, attention/executive function, and visuospatial skills, consistent with previous literature.[8–11] Also in line with earlier studies,[9] performance in the Free and Cued Selective Reminding Test (FCSRT) subtests was the most affected by Aβ.

Figure S2 illustrates the demographic composition and representativeness of the normative dataset.

3.2 | NGN

Our GAMLSS-based approach revealed statistically significant effects of demographic variables across multiple distribution parameters (Table 3).

For the location parameter (μ), which represents the central tendency of the distribution for the outcome variable, higher age and lower educational level were significantly associated with overall

TABLE 2 Baseline demographics and neuropsychological performance of CN participants stratified by Aβ status.

Characteristics	Aβ− CN $n = 774$*	Aβ+ CN $n = 213$*	p value†	Effect size‡
Demographics				
Age (years)	60 (8.8)	67 (6.9)	**< 0.001**	$d = 0.83$
Education (years)	14 (4.2)	13 (4.5)	**0.004**	$d = 0.23$
Biological sex (female)	475 (61%)	119 (56%)	0.2	$\varphi c = 0.03$
Neuropsychological performance (*w*-scores)				
MMSE	0.0 (1.0)	0.0 (1.2)	0.6	$d = 0.04$
VOSP number location	0.0 (1.0)	−0.3 (1.2)	**0.005**	$d = 0.27$
ROCF copy	0.0 (1.0)	0.1 (1.1)	0.5	$d = 0.05$
FCSRT total free recall	0.0 (1.0)	−0.2 (1.1)	**0.035**	$d = 0.21$
FCSRT total recall	0.0 (1.0)	−0.4 (1.4)	**0.002**	$d = 0.36$
FCSRT delayed free recall	0.0 (1.0)	−0.4 (1.3)	**0.002**	$d = 0.34$
FCSRT delayed total recall	0.0 (1.0)	−0.4 (1.7)	**0.003**	$d = 0.38$
ROCF delayed recall	0.0 (1.0)	0.0 (1.1)	0.8	$d = 0.02$
TMT-A	0.0 (1.0)	−0.2 (1.3)	**0.04**	$d = 0.19$
TMT-B	0.0 (1.0)	−0.2 (1.2)	**0.014**	$d = 0.22$
Digit Span Forward	0.0 (1.0)	0.1 (1.0)	0.3	$d = 0.09$
Digit Span Backward	0.0 (1.0)	−0.1 (0.9)	0.052	$d = 0.15$
Phonetic fluency	0.0 (1.0)	0.0 (1.0)	0.7	$d = 0.03$
Semantic fluency	0.0 (1.0)	0.0 (1.0)	> 0.9	$d = 0$
BNT	0.0 (1.0)	−0.1 (1.2)	0.5	$d = 0.05$

Note: Descriptive and inferential statistics on baseline demographics and neuropsychological performance of conventionally diagnosed CN participants, stratified by Aβ status. Raw neuropsychological scores were converted to demographically adjusted *w*-scores (reference group: Aβ− CN) to account for the potential influence of demographic variables on neuropsychological performance and to facilitate comparison across measures. Bold *p* values indicate statistical significance (< 0.05).

Abbreviations: Aβ, amyloid β; Aβ−, Aβ-negative; Aβ+, Aβ-positive; BNT, Boston Naming Test; CN, cognitively normal; FCSRT, Free and Cued Selective Reminding Test; MMSE, Mini-Mental State Examination; ROCF, Rey-Osterrieth Complex Figure; SD, standard deviation; TMT-A/B, Trail Making Test Part A/Part B; VOSP, Visual Object and Space Perception Battery.

*Mean (SD); *n* (%).

†Welch's two sample *t*-test; Pearson's chi-squared test.

‡*d* = Cohen's d, φc = Cramér's V.

poorer performance on all neuropsychological measures except the Visual Object and Space Perception Battery (VOSP) number location test and the Boston Naming Test (BNT), for which no significant associations were found. For the Rey-Osterrieth Complex Figure (ROCF) copy, Trail Making Test Part A (TMT-A), Trail Making Test Part B (TMT-B), Digit Span Forward, and semantic fluency, these associations were moderated by complex and non-linear relationships (which were modeled by polynomials and P-splines) that caused variations in the magnitude and direction of change at different values of the predictor variables. Sex was identified as a significant predictor of the μ parameter for seven of the 14 tests analyzed, with females outperforming males in the FCSRT total free recall; conversely, a male advantage was observed in the ROCF delayed recall, TMT-A, TMT-B, Digit Span Forward, Digit Span Backward, and semantic fluency.

The scale parameter (σ), which represents the dispersion of the distribution for the response variable, showed a positive relationship with age for the FCSRT total recall, FCSRT delayed free recall, FCSRT delayed total recall, TMT-A, and TMT-B, indicating increased variability in cognitive performance among older individuals. In contrast, we observed a negative relationship between the σ parameter and age for the BNT, indicating decreased variability with increasing age. Higher educational level was associated with greater variability in cognitive performance for the VOSP number location and Digit Span Backward; contrarily, higher levels of education were associated with decreased variability for the ROCF copy and TMT-B. Male sex was associated with greater variability in cognitive performance for the Digit Span Forward and Digit Span Backward.

For the shape parameter ν, which controls skewness, we observed that higher age and lower educational level were significantly associated with greater negative skewness of the BNT distribution.

Finally, for the shape parameter τ, controlling kurtosis, higher age was associated with a more peaked distribution of the TMT-B scores.

Age-, education-, and sex-adjusted *z*-scores for all neuropsychological measures included in this study are available in an open-access ShinyApp (https://cogstats.shinyapps.io/cogstats/).

TABLE 3 Statistically significant predictors in GAMLSS models.

Neuropsychological test	Distribution parameter	Term	Estimate	Std. error	*p* value
VOSP number location	μ	Intercept	2.43	0.06	< 0.001
	σ	Intercept	−2.66	0.2	< 0.001
		Education	14.85	5	< 0.01
ROCF copy	μ	Intercept	2.14	0.04	< 0.001
		Age (linear)	−1.91	0.86	< 0.05
		Age (quadratic)	−2.78	0.8	< 0.001
		Education	6.73	0.96	< 0.001
	σ	Intercept	−2.54	0.07	< 0.001
		Education	−6.01	1.83	< 0.01
FCSRT total free recall	μ	Intercept	37.23	1.73	< 0.001
		Age	−0.21	0.02	< 0.001
		Education	0.24	0.05	< 0.001
		Sex (male)	−1.74	0.43	< 0.001
	σ	Intercept	1.69	0.03	< 0.001
FCSRT total recall	μ	Intercept	2.35	0.04	< 0.001
		Age	−5.74	1.05	< 0.001
		Education	4.9	0.93	< 0.001
	σ	Intercept	−2.79	0.08	< 0.001
		Age	8.15	2.28	< 0.001
FCSRT delayed free recall	μ	Intercept	0.81	0.03	< 0.001
		Age	−6.41	0.77	< 0.001
		Education	3.27	0.75	< 0.001
	σ	Intercept	−2.9	0.13	< 0.001
		Age	9.76	3.26	< 0.01
FCSRT delayed total recall	μ	Intercept	3.18	0.06	< 0.001
		Age	−10.86	1.77	< 0.001
		Education	7.31	1.5	< 0.001
	σ	Intercept	−3.18	0.21	< 0.001
		Age	14.31	5.52	< 0.01
ROCF delayed recall	μ	Intercept	21.37	1.74	< 0.001
		Age	−0.17	0.02	< 0.001
		Education	0.3	0.05	< 0.001
		Sex (male)	1.89	0.42	< 0.001
	σ	Intercept	1.66	0.03	< 0.001
TMT-A	μ	Intercept	38.31	0.59	< 0.001
		Age (linear)	173.32	14.03	< 0.001
		Age (quadratic)	57.65	11.81	< 0.001
		Education (linear)	−95.34	13.9	< 0.001
		Education (quadratic)	69.91	16.37	< 0.001
		Education (cubic)	−34.34	15.6	< 0.05
		Sex (male)	−3	0.78	< 0.001

(Continues)

TABLE 3 (Continued)

Neuropsychological test	Distribution parameter	Term	Estimate	Std. error	*p* value
	σ	Intercept	−1.16	0.03	< 0.001
		Age (linear)	1.99	0.74	< 0.01
	ν	Intercept	−0.08	0.09	0.38
	τ	Intercept	0.68	0.08	< 0.001
TMT-B	μ	Intercept	87.86	1.71	< 0.001
		Age (linear)	345.48	36.64	< 0.001
		Age (quadratic)	152.62	74.63	< 0.05
		Education (linear)	−488.53	51.04	< 0.001
		Education (quadratic)	373.6	65.11	< 0.001
		Education (cubic)	−191.29	52.56	< 0.001
		Sex (male)	−5.59	2.01	< 0.01
	σ	Intercept	−1	0.03	< 0.001
		Age (linear)	2.18	0.83	< 0.01
		Education (linear)	−2.71	0.76	< 0.001
	ν	Intercept	−0.33	0.08	< 0.001
	τ	Intercept	0.53	0.09	< 0.001
		Age (linear)	6.04	2.16	< 0.01
Digit Span Forward	μ	Intercept	0.79	0.18	< 0.001
		s(Age)	−0.01	0	< 0.001
		s(Education)	0.01	0.01	< 0.01
		Sex (male)	0.18	0.04	< 0.001
	σ	Intercept	−0.57	0.07	< 0.001
		Sex (male)	0.29	0.1	< 0.01
Digit Span Backward	μ	Intercept	0.2	0.03	< 0.001
		Age	−2.43	0.59	< 0.001
		Education	5.23	0.58	< 0.001
		Sex (male)	0.14	0.04	< 0.01
	σ	Intercept	−0.59	0.06	< 0.001
		Education	7.17	1.29	< 0.001
		Sex (male)	0.27	0.1	< 0.01
Phonetic fluency	μ	Intercept	17.02	1.41	< 0.001
		Age	−0.11	0.02	< 0.001
		Education	0.4	0.04	< 0.001
	σ	Intercept	1.48	0.03	< 0.001
Semantic fluency	μ	Intercept	28.07	1.46	< 0.001
		s(Age)	−0.19	0.02	< 0.001
		s(Education)	0.32	0.04	< 0.001
		Sex (male)	1.22	0.38	< 0.01
	σ	Intercept	−1.4	0.03	< 0.001
	ν	Intercept	0.68	0.12	< 0.001
	τ	Intercept	0.55	0.08	< 0.001

(Continues)

TABLE 3 (Continued)

Neuropsychological test	Distribution parameter	Term	Estimate	Std. error	*p* value
BNT	μ	Intercept	90.18	0.8	< 0.001
	σ	Intercept	−1.47	0.44	< 0.001
		s(Age)	−0.01	0.01	< 0.05
	ν	Intercept	20.96	4.68	< 0.001
		s(Age)	−0.22	0.06	< 0.001
		s(Education)	0.28	0.1	< 0.01
	τ	Intercept	1.26	0.21	< 0.001

Note: Intercept and estimates for the statistically significant predictor variables age, education, and sex (reference category: male) on applicable GAMLSS model parameters (i.e., μ: location; σ: scale; ν and τ: shape) across all neuropsychological tests. The parameters used depend on the distribution chosen for each test's model. The BB distribution was used for the VOSP number location, ROCF copy, FCSRT total recall, FCSRT delayed free recall, and FCSRT delayed total recall; the BCPE distribution was employed for the TMT-A, TMT-B, semantic fluency, and BNT; the DBI distribution was used for the Digit Span Forward and Digit Span Backward; the NO distribution was used for the FCSRT total free recall, ROCF delayed recall, and phonetic fluency. The distribution parameters were modeled using the following link functions: μ was modeled using the identity link function for BCPE and NO distributions, and the logit link function for BB and DBI; σ, ν, and τ parameters were modeled using log, identity, and log link functions, respectively. We used smoothing terms (i.e., polynomials and P-splines) to account for non-linear relationships between continuous predictors, age and education, and response variables. "Predictor (linear/quadratic/cubic)" refers to the degree of the polynomial term; "s(predictor)" refers to a smoothing function implemented using P-splines. Positive estimates indicate an increase in the respective parameter. Care should be taken in interpreting the sign of the estimates for the TMT-A/B, as higher scores in these measures indicate poorer performance (i.e., longer time to completion).

Abbreviations: BB, beta-binomial; BCPE, Box-Cox power exponential; BNT, Boston Naming Test; DBI, double-binomial; FCSRT, Free and Cued Selective Reminding Test; GAMLSS, generalized additive models for location, scale, and shape; NO, Normal; ROCF, Rey-Osterrieth Complex Figure; Std. error, standard error; TMT-A/B, Trail Making Test Part A/Part B; VOSP, Visual Object and Space Perception Battery.

4 | DISCUSSION

In this multicenter study, we applied the GAMLSS framework to derive comprehensive neuropsychological norms from 774 Aβ− CN individuals aged 30 to 90 years. To the best of our knowledge, this is the first study to provide biomarker-negative norms using GAMLSS.

NGN were adjusted for age, education, and sex. Across the multiple neuropsychological variables analyzed, we found that different demographic predictors exerted statistically significant effects on distinct distribution parameters (i.e., μ, σ, ν, τ). The subsequent discussion provides an overview of the key similarities and differences with prior studies; however, a detailed test-by-test comparative analysis is beyond the scope of this work. Regarding the impact of age and education on mean cognitive performance, our results are broadly consistent with previous Spanish normative studies.[22,23] In particular, although we found that the effects of age and education were non-linear for some measures, the overall pattern indicates that increasing age and lower education were independently associated with a decline in expected mean performance across all the neuropsychological tests, except for the VOSP number location and the BNT. Concerning the VOSP number location, the only demographic variable found to have a statistically significant effect on any distribution parameter was education, which was associated with increased variability in performance among highly educated individuals. Our results are in agreement with previous Spanish normative data, which found that education accounted significantly for the variance in raw scores of the VOSP number location, while the age effect was negligible.[24] As to the BNT, we found no evidence for a direct effect of age or education on the mean performance, which diverges from what has been reported in earlier studies[32–34]; however, we found that age was associated with a decrease in the skewness of the distribution (i.e., among older participants, a greater proportion of individuals showed poorer performance in the BNT, thus lowering the mean), while education was associated with an increase in skewness (i.e., among highly educated participants, more individuals exhibited higher performance in the BNT, thus increasing the mean). The discrepancy between our results and previous studies may reflect the greater precision and flexibility provided by GAMLSS in modeling all distribution parameters beyond the mean.

Finally, unlike previous Spanish normative studies that provided age- and education-specific norms but did not detect relevant sex differences in any of the tests considered in the present study,[22,23] we observed that sex had a statistically significant effect on the performance across seven of the 14 tests analyzed, and was therefore incorporated into their models. Sex influenced key cognitive domains, including memory, attention/executive function, and language. Regarding memory, we observed that females outperformed males in the FCSRT total free recall, while males outperformed women in the ROCF delayed recall. Our findings are in line with those of previous studies reporting a female advantage in verbal memory and a male advantage in visual memory.[11,13,41,42] We also found a male advantage in the TMT-A, TMT-B, Digit Span Forward, and Digit Span Backward. These results are largely in agreement with those of a recent article suggesting that men outperform women in all of these tasks except for the TMT-B, for which they found no relevant sex differences.[14] Finally, our results point toward a male advantage in semantic fluency (animals). This finding is consistent with previous literature suggesting that sex differences in semantic fluency depend on the semantic category, with males demonstrating better performance in naming animals, and

females showing better performance in naming fruits, food, vegetables, and kitchen tools.[30,41]

This study has several strengths. First, NGN were derived from a large Aβ− normative sample. This approach is supported by prior literature demonstrating that Aβ affects performance in CN individuals,[8–11] and is further validated by our findings. Specifically, in line with previous studies, we observed that Aβ+ CN individuals showed a small but significant cognitive impairment in verbal memory (all subtests of the FCSRT: p = 0.002 to 0.035, d = 0.21 to 0.38),[8,9,11] attention/executive function (TMT-A and TMT-B: p = 0.04 and 0.014, d = 0.19 and 0.22, respectively),[8,11] and visuospatial skills (VOSP number location: $p < 0.005$, $d = 0.27$).[8] Also consistent with prior studies, we found no statistically significant Aβ+-related impairment in the Digit Span Forward or Backward ($p = 0.3$ and 0.052, $d = 0.09$ and 0.15, respectively),[8,10] phonetic fluency (p = 0.7, d = 0.03),[8] semantic fluency ($p > 0.9$, $d = 0$),[8,11] or BNT ($p = 0.5$, $d = 0.05$).[8,10] Regarding the ROCF test, our results are partially consistent with prior findings:[10] while the absence of a statistically significant difference in the ROCF copy (p = 0.5, d = 0.05) aligns with previous results, we also did not observe a significant difference in the ROCF delayed recall (p = 0.8, $d = 0.02$). Finally, our analysis revealed no statistically significant Aβ+-related impairment in global cognition (MMSE: $p = 0.6$, $d = 0.04$), which aligns with some previous studies,[9] while other studies using different measures of global cognition have reported differing results (Table 2).[8] Second, we believe that the use of GAMLSS constitutes a methodological strength, and we expect them to have contributed to obtaining reliable normative data; however, definitive evidence of the diagnostic accuracy of NGN is yet to be established in future studies. Finally, we anticipate that adjusting NGN for sex, along with age and education, is likely to enhance their practical value.[43]

Despite the strengths of this study, some limitations should be considered. First, although our large multicenter sample was broadly representative of the Spanish population in terms of age, education, and sex, it may not fully capture the diversity of other Spanish-speaking groups due to the lack of stratification by ethnicity or cultural background. Second, a potential circularity bias may have arisen because several neuropsychological measures used for diagnostic classification of CN individuals were later included in the normative analyses (see Table S1 and Table 1). Although classification of participants as CN was also informed by independent tests, functional assessments, and clinical judgment, this partial overlap may have undermined the validity of NGN. Alternative strategies for normative sample selection–such as relying solely on cognitive and/or functional screening instruments,[22,44] applying consensus diagnoses blinded to formal neuropsychological performance,[15,45] or employing robust normative sampling that excludes individuals who subsequently develop cognitive decline during longitudinal follow-up[46]–avoid such overlap, but have their own practical and methodological constraints (e.g., ceiling effects and insensitivity to subtle or domain-specific deficits,[47,48] subjectivity,[49] and reduced sample sizes resulting from long-term monitoring[50]). Our approach may represent a balance between practical feasibility and methodological rigor. Third, while NGN excluded individuals with Aβ pathology, other neuropathologies (e.g., α-synuclein, TDP-43) could not be ruled out, potentially biasing estimates of normal cognition. Fourth, the neuropsychological battery, while comprehensive, did not include measures of social cognition. However, the selected tests reflect those most widely used in clinical settings, facilitating the implementation of NGN. Future studies may potentially overcome these limitations by incorporating additional neuropsychological tests, broader biomarker panels, and more diverse normative populations.

Here, we derived GAMLSS-based comprehensive neuropsychological norms from a large multicenter sample of AD-negative CN adults. Future research is warranted to evaluate their utility in cognitive classification relative to current standards.

ACKNOWLEDGMENTS

We express our gratitude to all participants of this study and all the clinical and research teams involved in their assessment. Alexandre Bejanin acknowledges support from Instituto de Salud Carlos III (ISCIII) and co-funded by the European Union through the Miguel Servet grant (CP20/00038), and the Alzheimer's Association (AARG-22-923680). Mircea Balasa reported receiving support from ISCIII (PI22/00343). Javier Arranz was supported by ISCIII through the Río Hortega Fellowship CM21/00243 and co-funded by the European Union. Nuole Zhu was supported by contract CM21/00113 from ISCIII, co-funded by the European Union. Juan Fortea reports grants from Fundación Tatiana Pérez de Guzmán el Bueno (IIBSP-DOW-2020-151). He was also supported by Horizon2020–Research and Innovation Framework Programme from the European Union (H2020-SC1-BHC-2018-2020). Raquel Sánchez-Valle reported receiving funding from ISCIII (20/00448). Pablo Martínez-Lage reported receiving funding from the Department of Economic Promotion of the Provincial Government of Gipuzkoa (124/16), the Department of Health of the Basque Government (2016111096, S-PR12CH001, S-PR13ZH001), and ISCIII (PI12/02262, PI15/00919). Ignacio Illán-Gala was supported by the Juan Rodés Contract (JR20/0018) from ISCIII, partly funded by the European Social Fund. He is a senior Atlantic Fellow for Equity in Brain Health at the Global Brain Health Institute (GBHI) and receives funding from the Alzheimer's Association (AACSF-21-850193), and the Alzheimer Society (GBHI ALZ UK-21-72097). The SPIN cohort received funding from Fondo de Investigaciones Sanitario, ISCIII (PI13/01532, PI14/01126, PI16/01825, PI17/01019, PI17/01896, PI18/00335, PI18/00435, PI19/00882, PI20/01473, PI20/00836, PI21/01395, PI21/00063, PI21/00791, PI22/00611, PI22/00307, PI23/01786, PI24/00598, PI24/00968, PI24/01087, INT19/00016, INT21/00073, INT23/00048, AC19/00103, DTS22/00111) and the CIBERNED program (Program 1, Alzheimer Disease), jointly funded by Fondo Europeo de Desarrollo Regional, Unioń Europea, "Una manera de hacer Europa". The SPIN cohort was also supported by the National Institutes of Health grants (1R01AG056850-01A1, R21AG056974, R01AG061566, 1R01AG081394-01, 1R61AG066543-01, and R01AG080470), by Generalitat de Catalunya (2017-SGR-547, 2021-SGR-00979, SLT006/17/125, SLT006/17/119, SLT002/16/408), Fundació La Marató de TV3 (20141210, 044412, and 20142610), Ajuntament de Barcelona in collaboration with Fundació 'la Caixa'

(23S06157-001), Fundació Catalana Síndrome de Down, and Fundació Víctor Grífols i Lucas. Horizon 21 Consortium is partly funded by Jérôme Lejeune Foundation. The GAP study was supported by CITA-Alzheimer Foundation, ISCIII (PI12/02262 and PI1500919), and the Department of Health of the Basque Government (S-PR12CH001 and S-PR13ZH001). The DEBA project was partially funded by the Department of Economic Promotion of the Provincial Government of Gipuzkoa (2016 Support Programme for the Gipuzkoa Network for Science, Technology, and Innovation) and the Department of Health of the Basque Government (2016111096).

CONFLICT OF INTEREST STATEMENT

Sara Rubio-Guerra reported honoraria for educational events from Esteve Pharmaceuticals, S.A. Mircea Balasa reported receiving travel support for attending meetings from Lilly and serving on an advisory board for Novo Nordisk. Miren Altuna reported receiving honoraria for educational events from Lilly, Esteve, ITAMAR, Almirall, and KERN Pharma; as well as support for attending meetings or travel from Lilly, Esteve, and Almirall. Javier Arranz reported receiving honoraria for educational events from Roche, Esteve, and Lilly, and support for attending meetings or travel from Esteve Pharmaceuticals, S.A. Daniel Alcolea reported receiving consulting fees from Grifols S.A., Lilly, Fujirebio-Europe, and Roche Diagnostics; honoraria for educational events from Fujirebio-Europe, Roche Diagnostics, Nutricia, Krka Farmacéutica S.L., Zambon S.A.U., Esteve Pharmaceuticals, S.A., Neuraxpharm, Alter, and Lilly; and support for attending meetings or travel from Fujirebio-Europe, Lilly, and Nutricia. Daniel Alcolea also participated in advisory boards for Grifols S.A., Lilly, Fujirebio-Europe, and Roche Diagnostics. Alberto Lleó reported receiving consulting fees from Eisai, Esteve, Fujirebio-Europe, Roche, Grifols S.A., and Lilly. Juan Fortea reported receiving consulting fees from Lundbeck, Ionis, and AC Immune; honoraria for educational events from Roche, Esteve, Biogen, Laboratorios Carnot, Adamed, Life Molecular Imaging, Eisai, and Lilly. Juan Fortea has participated in advisory boards for AC Immune, Alzheon, Zambon, Lilly, Roche, Eisai, and Perha. Juan Fortea has held leadership or fiduciary roles in the Spanish Neurological Society, T21 Research Society, Lumind Foundation, Jérôme-Lejeune Foundation, Alzheimer's Association, Health Research Board, and Dementia Trials Ireland (all without payments). Juan Fortea also participated in Study Sections for the European Commission, National Institutes of Health, and Instituto de Salud Carlos III, with payments received for these activities. Juan Fortea reports receipt of equipment, materials, or other services from Life Molecular Imaging, provided to his institution. Daniel Alcolea, Alberto Lleó, and Juan Fortea declare a filed patent application (WO2019175379 A1 Markers of synaptopathy in neurodegenerative disease). Raquel Sánchez-Valle reported receiving consulting fees from Lilly (paid to her institution) and UCB (paid personally). Pablo Martínez-Lage reported receiving consulting fees from Lilly, Roche, and Eisai; honoraria for educational events from Lilly, Roche, Eisai, Nutricia, and Almirall; and support for attending meetings or travel from Lilly, Roche, Eisai, and Nutricia. Ignacio Illán-Gala reported receiving honoraria for educational events from Esteve, Sociedad Española de Neurología, Societat Catalana de Neurologia, Lilly, Kern Pharma, and Almirall; support for attending meetings or travel from Esteve and Almirall; and participation in advisory boards for UCB and Nutricia. The remaining authors declare no conflicts of interest. Author disclosures are available in the supporting information.

CONSENT STATEMENT

Written informed consent was obtained from all human subjects before enrollment in the study.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Rubio-Guerra S, Sánchez-Saudinós MB, Sala I, et al. Development of amyloid-negative neuropsychological norms using GAMLSS. *Alzheimer's Dement*. 2025;17:e70224. https://doi.org/10.1002/dad2.70224

5.2. Article 2: Amyloid-negative neuropsychological norms: Added value in the era of biomarkers and disease-modifying therapies

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Received: 11 September 2025 | Accepted: 31 October 2025

DOI: 10.1002/dad2.70223

Alzheimer's & Dementia
Diagnosis, Assessment & Disease Monitoring

RESEARCH ARTICLE

Amyloid-negative neuropsychological norms: Added value in the era of biomarkers and disease-modifying therapies

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Abstract

INTRODUCTION: We previously applied generalized additive models for location, scale, and shape to derive amyloid β–negative next-generation norms (NGN) for a comprehensive neuropsychological battery. Here, we evaluated the accuracy of NGN in detecting cognitive impairment compared to traditional norms (TN).

METHODS: This multicenter study included $N = 2405$ participants classified as cognitively normal (CN, $n = 987$) or with mild cognitive impairment (MCI, $n = 1418$) using conventional criteria. All participants underwent neuropsychological testing and cerebrospinal fluid Alzheimer's disease (AD) biomarker assessment. We used actuarial neuropsychological criteria to reclassify all participants using TN and NGN.

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Department of Health of the Basque Government; European Union; Fondo Europeo de Desarrollo Regional; Fundació Catalana Síndrome de Down; Fundació 'la Caixa'; Fundació La Marató de TV3; Fundación Tatiana Pérez de Guzmán el Bueno; Fundació Víctor Grífols i Lucas; Generalitat de Catalunya; Global Brain Health Institute; Instituto de Salud Carlos III; Jérôme Lejeune Foundation; National Institutes of Health

Diagnostic groups were compared on cognitive performance, AD biomarker positivity, and longitudinal cognitive trajectories.

RESULTS: Nineteen percent of TN-classified CN participants were diagnosed with MCI by NGN, whereas 3% of TN-classified MCI were identified as CN by NGN. NGN demonstrated stronger associations with neuropsychological performance, AD biomarkers, and progression than TN.

DISCUSSION: NGN enhance the detection of objective cognitive impairment, with direct implications for clinical practice and research.

KEYWORDS

Alzheimer's disease, biomarkers, clinical trials, cognitive trajectories, diagnosis, disease-modifying therapies, MCI, neuropsychological norms, normative data

Highlights

- Next-generation norms (NGN) reclassify one of every five cases from cognitively normal (CN) to mild cognitive impairment (MCI).
- This group shows poor cognitive performance and a high prevalence of amyloid β positivity.
- NGN-based diagnosis of MCI predicts cognitive progression on follow-up.
- Results indicate that NGN improve the detection of objective cognitive impairment.
- NGN can inform biomarker use, therapy indication, and clinical trial design.

1 | BACKGROUND

Alzheimer's disease (AD) is the leading cause of dementia;[1] yet, the majority of individuals on the AD continuum are in the predementia stages, including the preclinical AD (i.e., positive AD biomarkers, but performance in the normal range on objective cognitive assessment) and prodromal AD (i.e., mild cognitive impairment [MCI] due to AD) stages.[2] The recent approval of the first disease-modifying drugs for AD, targeting amyloid β (Aβ) plaques in individuals at early symptomatic stages,[3–5] underscores the importance of timely, biomarker-confirmed AD diagnosis, as these therapies show the greatest benefit in earlier disease stages.[6,7] At the same time, in the absence of effective preventive treatments, biomarkers are not recommended for use in cognitively normal (CN) individuals in clinical practice.[8,9] Accurate neuropsychological norms are therefore critical to identify the earliest objectively measurable cognitive deficits that inform the use of biomarkers and the selection of patients for therapeutic interventions.

We recently developed comprehensive neuropsychological normative data, referred to as next-generation norms (NGN).[10] NGN incorporated two methodological advancements: (1) the selection of a normative sample consisting of CN individuals without biomarker evidence of Aβ accumulation, and (2) the modeling of normative data under the generalized additive models for location, scale, and shape (GAMLSS). Norms were adjusted for age, education, and sex.

In the present follow-up study, we aim to evaluate the accuracy of NGN in detecting cognitive impairment compared to traditional norms (TN). However, direct comparison of neuropsychological norms is challenged by the lack of both uniform test batteries and an operationalized definition of MCI.[11,12] Conventional MCI criteria rely on subjective cognitive decline, clinical judgment based on cognitive and functional screening tools, and impairment in one or more neuropsychological tests,[13,14] which may result in false-positive and false-negative diagnostic errors.[15,16] In contrast, actuarial MCI criteria such as the Jak/Bondi approach, which require impairment on at least two measures within a cognitive domain,[17] have demonstrated greater diagnostic stability and stronger associations with AD biomarkers and progression.[18,19]

Accordingly, to compare NGN and TN, we selected a large multicenter dataset of individuals without dementia and applied actuarial neuropsychological criteria to cognitive scores based on both NGN and TN from the same test battery. We hypothesized that NGN would yield stronger associations with neuropsychological performance, AD biomarker positivity, and clinical progression than TN.

2 | METHODS

2.1 | Participants and design

Data were prospectively collected as part of the longitudinal multicenter SIGNAL study, which harmonized core neuropsychological procedures across several Spanish centers.[20] For the present study, we retrospectively selected N = 2405 individuals without dementia (CN: n = 987; MCI: n = 1418) recruited between March 2006 and

RESEARCH IN CONTEXT

1. Systematic Review: Current Spanish neuropsychological norms do not account for biomarker status and are based on traditional norming methods. Building upon accumulated knowledge from prior studies, we recently derived next-generation norms (NGN) from a large sample of amyloid β–negative cognitively normal individuals using generalized additive models for location, scale, and shape (GAMLSS).
2. Interpretation: Despite the theoretical advantages of NGN, evidence is needed to establish their actual added value. NGN-based cognitive classification demonstrated enhanced associations with neuropsychological performance, Alzheimer's disease biomarker positivity, and longitudinal cognitive trajectories compared to current normative standards. These results suggest that NGN enhance the detection of cognitive impairment and may therefore improve clinical and research outcomes.
3. Future Directions: Future research should provide further evidence to confirm the utility of NGN.

July 2022. Participants were recruited from both outpatient memory clinics and community-based volunteer populations. The inclusion criteria were: (1) absence of dementia, (2) age ≥ 30 years, and baseline availability of both (3) a neuropsychological evaluation and (4) cerebrospinal fluid (CSF) AD biomarker assessment, including Aβ status. The exclusion criterion was a history of neurological, psychiatric, or systemic conditions that might affect cognitive performance (Figure S1). Participants were classified at baseline as CN or MCI using conventional criteria[13,14] based only on clinical and neuropsychological data at the time of CSF collection, without regard to biomarker results or follow-up information. We previously derived NGN from a normative dataset comprising all Aβ-negative (Aβ–) CN individuals (n = 774).[10] This study adhered to the 1964 Declaration of Helsinki and was approved by the ethics committees of all participating centers. Written informed consent was obtained from all participants before enrollment.

2.2 | Materials and procedure

2.2.1 | Neuropsychological evaluation

All participants underwent a baseline neuropsychological evaluation according to their center's protocol (Table S1). The assessment incorporated the Mini-Mental State Examination (MMSE; range 0–30, with lower scores reflecting greater impairment) as a measure of general cognitive functioning.[21] The neuropsychological measures included in the present validation study were those for which NGN were derived in our prior study, and included at least two measures across the domains of: visuospatial skills (the Visual Object and Space Perception Battery [VOSP] number location subtest and the Rey-Osterrieth Complex Figure [ROCF] copy), memory (the Free and Cued Selective Reminding Test [FCSRT] total free recall, FCSRT total recall, FCSRT delayed free recall, FCSRT delayed total recall, and the ROCF delayed recall), attention/executive function (the Trail Making Test Part A [TMT-A], Trail Making Test Part B [TMT-B], Digit Span Forward, Digit Span Backward, and phonetic fluency [words beginning with P]), and language (semantic fluency [animal names] and the Boston Naming Test [BNT]).

Raw neuropsychological scores of all participants (N = 2405) were converted into demographically adjusted normed scores according to NGN and published Spanish normative data (the Neuronorma Project[22,23]—from this point forward, "TN" will specifically refer to this set of traditional neuropsychological norms). Data were missing for 10% of the observations, with copy and delayed recall of the ROCF each accounting for 37% of the overall missing data.

2.2.2 | CSF biomarkers of AD

All participants underwent a baseline lumbar puncture to analyze CSF AD biomarkers, of which Aβ 1–42 (Aβ42) was the most widely available Aβ biomarker across centers. Individuals were classified as either Aβ-positive (Aβ+) or Aβ– based on each center's cutoff for Aβ42 (Supplementary Methods).

2.2.3 | Cognitive trajectories

In a subset of participants from Hospital de la Santa Creu i Sant Pau for whom at least a 1-year longitudinal neuropsychological assessment was available (n = 812), follow-up changes in the MMSE score were used to track their cognitive performance over time.

2.3 | Added value of NGN

To explore the potential added value of NGN over TN, we examined if NGN-derived cognitive status classification (i.e., CN or MCI) improved the associations with neuropsychological performance, CSF AD biomarkers, and cognitive trajectories compared to TN.

To compare the norms, we applied the same actuarial neuropsychological criteria, adapted from Jak/Bondi criteria,[17] to both NGN- and TN-based scores. Adaptation of Jak/Bondi criteria involved defining impairment as a performance < −1.5 standard deviations (SD) from the normative mean (corresponding to a z-score < −1.5 and a scaled score ≤ 5), as opposed to < −1 SD put forward by Jak/Bondi. This midpoint cutoff (−1.5 vs. −1 or −2 SD) aligns with common practices in the field and offers a good compromise between sensitivity and specificity. Participants were actuarially diagnosed with MCI if they had two or more impaired normed scores within at least one cognitive domain across memory, attention/executive function, or language. Participants were considered CN only if they had at least two cognitive measures available across all the examined domains, and the criteria for MCI were not

met. In making actuarial diagnoses, we did not account for visuospatial function, as 47% of the participants had insufficient data available for classification in this domain; thus, considering visuospatial skills would have precluded many otherwise CN individuals from receiving a neuropsychological diagnosis.

Four possible combinations of actuarial neuropsychological diagnoses were expected: individuals with a normal performance according to both TN and NGN (CN_{tn}/CN_{ngn}); individuals performing abnormally by both norms (MCI_{tn}/MCI_{ngn}); individuals classified as CN based on TN, but diagnosed with MCI under NGN (CN_{tn}/MCI_{ngn}); and those classified as MCI according to TN, but performing normally as per NGN (MCI_{tn}/CN_{ngn}). We explored the frequencies of these diagnostic groups and conducted between-group comparisons on their demographic characteristics, neuropsychological performance, and rates of AD-related biomarkers. We also compared cognitive trajectories across diagnostic groups, as measured by changes in MMSE scores over time: we first analyzed the complete longitudinal dataset; subsequently, we performed separate subanalyses for Aβ+ and Aβ– participants to capture cognitive progression driven by AD and non-AD underlying etiologies, respectively.

2.4 | Statistical analyses

Statistical analyses were conducted using R, version 4.2.3.[24] Between-group differences in continuous variables were assessed using *t*-tests for two-group comparisons and one-way analysis of variance (ANOVA) for three or more groups. For unequal variances, we applied Welch's *t*-test and Welch's ANOVA. Group differences in categorical variables were examined using chi-squared tests. Statistical significance for all tests was set at p value < 0.05 ($\alpha = 0.05$). Standardized effect size statistics were calculated to quantify the magnitudes of between-group differences: we reported Cohen's d (d) for *t*-tests, eta-squared (η^2) for ANOVA, and Cramér's V (φc) for chi-squared tests. The magnitudes of the differences were interpreted as small, medium, or large based on generally accepted thresholds.[25] To account for demographic influences when comparing neuropsychological performance across diagnostic groups, we calculated *w*-scores (standardized residuals) using a fully adjusted regression approach. For each neuropsychological measure, a multiple linear regression model was fitted in the CN_{tn}/CN_{ngn} reference group. Based on clinical knowledge, age, education, and sex were included as covariates in all models, irrespective of their univariate or multivariate significance. The predicted value for each participant was subtracted from their observed raw score and divided by the model's residual SD, yielding a *w*-score that represents individual deviation from demographically adjusted expected performance. Post hoc analyses were corrected for multiple comparisons using the Bonferroni method. To conduct difference and equivalence tests, we prespecified thresholds for a meaningful difference (δ): ≥ 0.20 for d, ≥ 0.01 for η^2, and ≥ 0.10 for φc, according to conventions.[25] We concluded that differences in between-group comparisons existed whenever a statistically significant and meaningful difference was found. Equivalence between groups was established when the upper limit of the 90% (1–2α) confidence interval for the effect size fell entirely below the predefined equivalence threshold δ.[26] We applied linear mixed-effects (LME) models for the longitudinal analyses to predict annual changes in MMSE scores across diagnostic groups. The models incorporated fixed effects for the group (up to four levels: CN_{tn}/CN_{ngn}, MCI_{tn}/MCI_{ngn}, CN_{tn}/MCI_{ngn}, and MCI_{tn}/CN_{ngn}), time, and their interaction. Age at baseline, education, and sex were also incorporated as fixed effects to account for their potential influence on cognitive trajectories. To consider individual variability in baseline scores and rates of change over time, a random intercept, as well as a random slope for time, were included for each participant. We used the restricted maximum likelihood method for model estimation. Akaike Information Criterion was used to assess our final models' fit.

3 | RESULTS

3.1 | Demographics and baseline characteristics

Table 1 presents baseline characteristics of all participants, stratified by conventional diagnosis. The MCI group was older, had fewer years of education, lower MMSE scores, and a higher proportion of CSF Aβ+ participants than the CN group. The sex distribution was equivalent between the MCI and CN groups. Raw neuropsychological scores of MCI individuals were significantly worse than those of CN individuals on all 14 cognitive measures, with large effect sizes.

3.2 | Added value of NGN

Of the 2405 participants, 2185 had enough baseline neuropsychological data to receive an actuarial cognitive classification based on both TN and NGN. Among these, 81% of the individuals actuarially identified as CN by TN, and 97% of those diagnosed with MCI by TN, were classified the same by NGN (CN_{tn}/CN_{ngn} and MCI_{tn}/MCI_{ngn} groups, respectively). Conversely, 19% of TN-classified CN participants were diagnosed with MCI by NGN (CN_{tn}/MCI_{ngn}). Finally, 3% of TN-classified MCI individuals were considered CN by NGN (MCI_{tn}/CN_{ngn}; Figure 1). Table 2 displays baseline demographics, neuropsychological performance, and proportions of CSF Aβ+ individuals across the four actuarially reclassified diagnostic groups. Comparisons of TN- and NGN-derived actuarial classifications versus conventional diagnoses made at each center are provided in Tables S2 and S3.

3.2.1 | Demographic characteristics

Across the four diagnostic groups, statistically significant overall differences were observed for age and years of education, with effect sizes indicating a medium-to-large difference for age and a small-to-medium difference for education. No significant differences were observed for sex distribution (Table 2).

TABLE 1 Baseline demographic, neuropsychological, and biomarker characteristics of participants.

Characteristics	All participants $N = 2405$*	CN $n = 987$*	MCI $n = 1418$*	*p* value†	Effect size‡
Demographics					
Age (years)	67 (9.5)	62 (8.9)	70 (8.4)	**< 0.001**	*d* = 0.98
Education (years)	12 (4.8)	13 (4.3)	11 (4.8)	**< 0.001**	*d* = 0.50
Sex (female)	1367 (57%)	594 (60%)	773 (55%)	**0.007**	φc = 0.05
Clinical measures					
MMSE (/30)	27.0 (2.9)	28.7 (1.5)	25.8 (3.0)	**< 0.001**	*d* = 1.17
Neuropsychological performance (raw scores)					
VOSP number location (/10)	8.0 (2.3)	9.1 (1.2)	7.4 (2.5)	**< 0.001**	*d* = 0.80
ROCF copy (/36)	29.8 (6.2)	32.0 (3.6)	26.9 (7.5)	**< 0.001**	*d* = 0.90
FCSRT total free recall (/48)	17.2 (10.3)	26.7 (6.3)	11.4 (7.6)	**< 0.001**	*d* = 2.15
FCSRT total recall (/48)	33.0 (12.8)	43.3 (4.7)	26.6 (12.1)	**< 0.001**	*d* = 1.67
FCSRT delayed free recall (/16)	6.4 (4.8)	10.7 (2.9)	3.7 (3.6)	**< 0.001**	*d* = 2.10
FCSRT delayed total recall (/16)	11.3 (4.9)	15.1 (1.4)	8.9 (4.8)	**< 0.001**	*d* = 1.64
ROCF delayed recall (/36)	13.2 (7.0)	16.1 (5.9)	9.2 (6.4)	**< 0.001**	*d* = 1.14
TMT-A (s)	62.6 (36.1)	41.3 (18.4)	77.5 (37.9)	**< 0.001**	*d* = 1.16
TMT-B (s)	176.1 (98.8)	103.0 (63.5)	227.3 (86.1)	**< 0.001**	*d* = 1.60
Digit Span Forward (/9)	5.2 (1.2)	5.7 (1.2)	4.9 (1.1)	**< 0.001**	*d* = 0.75
Digit Span Backward (/8)	3.9 (1.2)	4.4 (1.2)	3.5 (1.1)	**< 0.001**	*d* = 0.88
Phonetic fluency (*n*)	12.2 (5.6)	15.8 (4.9)	9.6 (4.6)	**< 0.001**	*d* = 1.31
Semantic fluency (*n*)	16.3 (6.8)	21.1 (6.0)	12.9 (5.0)	**< 0.001**	*d* = 1.51
BNT (%)	81.7 (14.5)	90.5 (7.8)	75.6 (15.0)	**< 0.001**	*d* = 1.18
CSF Aβ status					
Aβ+	1008 (42%)	213 (22%)	795 (56%)	**< 0.001**	φc = 0.34

Note: Descriptive and inferential statistics on baseline demographics, MMSE scores, raw neuropsychological scores, and frequencies of CSF Aβ+ participants, stratified by conventional diagnosis. Bold *p* values indicate statistical significance (< 0.05).
Abbreviations: Aβ, amyloid β; Aβ+, Aβ–positive; AD, Alzheimer's disease; BNT, Boston Naming Test; CN, cognitively normal; CSF, cerebrospinal fluid; FCSRT, Free and Cued Selective Reminding Test; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; ROCF, Rey–Osterrieth Complex Figure; SD, standard deviation; TMT-A/B, Trail Making Test Part A/Part B; VOSP, Visual Object and Space Perception Battery.
*Mean (SD); *n* (%).
†Welch's two sample *t*-test; Pearson's chi-squared test.
‡*d* = Cohen's d, φc = Cramér's V.

3.2.2 | Neuropsychological performance

For most neuropsychological measures, visual inspection of results indicated a stepwise cognitive performance across the four diagnostic groups: participants in the CN_{tn}/CN_{ngn} group performed better than those in the MCI_{tn}/CN_{ngn} group, who in turn outperformed the CN_{tn}/MCI_{ngn} group; finally, the MCI_{tn}/MCI_{ngn} group showed the lowest performance. Inferential analyses confirmed significant and meaningful between-group differences across all neuropsychological measures after adjusting for relevant demographic factors, with the largest differences in FCSRT subtests (i.e., verbal memory measures; Table 2).

3.2.3 | CSF biomarkers of AD

A chi-squared test revealed significant between-group differences in CSF Aβ+ prevalence. The largest difference was observed between the CN_{tn}/CN_{ngn} (22%) and MCI_{tn}/MCI_{ngn} (65%) groups. Notably, the MCI_{tn}/CN_{ngn} group showed an Aβ+ prevalence (8.7%) statistically equivalent to that in the CN_{tn}/CN_{ngn} group, while the proportion of Aβ+ individuals in the CN_{tn}/MCI_{ngn} group (48%) more closely resembled that in the MCI_{tn}/MCI_{ngn} group (Table 2; Figure 2).

3.2.4 | Cognitive trajectories

Longitudinal data were available for 812 participants (CN_{tn}/CN_{ngn}, $n = 374$; MCI_{tn}/CN_{ngn}, $n = 8$; CN_{tn}/MCI_{ngn}, $n = 105$; MCI_{tn}/MCI_{ngn}, $n = 325$), representing 34% of the total sample. The longitudinal cohort consisted of 322 Aβ+ and 490 Aβ– participants. Significant between-group differences in baseline MMSE scores were observed, with a large effect size. The differences in baseline MMSE scores followed this pattern: $CN_{tn}/CN_{ngn} > MCI_{tn}/CN_{ngn} > CN_{tn}/MCI_{ngn} > MCI_{tn}/MCI_{ngn}$. Participants underwent a mean (SD) of 3.6 (1.9) MMSE assessments,

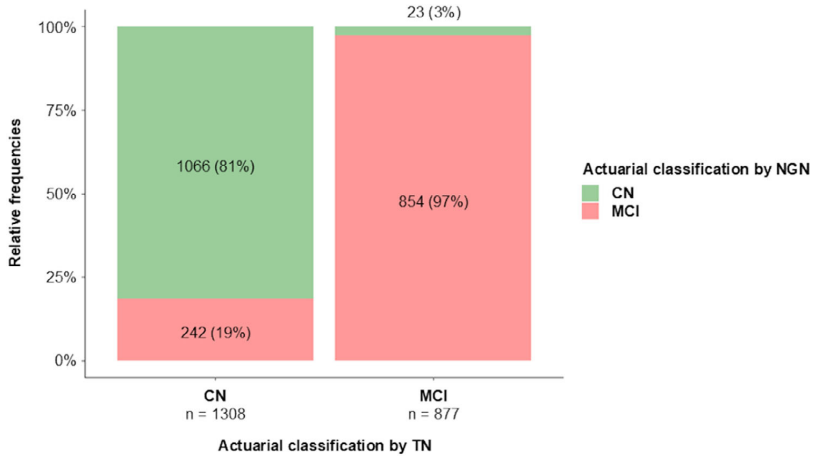


FIGURE 1 Cognitive classification by NGN versus TN. Absolute and relative frequencies of actuarial cognitive classifications based on NGN compared to those based on TN. CN, cognitively normal; MCI, mild cognitive impairment; NGN, next-generation norms; TN, traditional norms.

with a median (range) of 3 (2–11) evaluations, over a mean (SD) follow-up period of 4.2 (2.7) years, with a median (range) of 3.5 (1.0–14.1) years. Importantly, no statistically significant between-group differences were found in terms of follow-up number of visits or duration, enhancing the comparability of longitudinal cognitive trajectories across groups (Table S4).

We first applied an LME model to the complete longitudinal dataset to explore general trends. Next, we conducted subanalyses on the Aβ+ and Aβ− cohorts. The MCI_{tn}/CN_{ngn} group was not included in the models due to insufficient sample size ($n = 8$) for valid estimation.

When analyzing the complete longitudinal dataset, the LME model revealed statistically significant differences in baseline MMSE scores and their annual changes for the MCI_{tn}/MCI_{ngn} and the CN_{tn}/MCI_{ngn} groups compared to the reference CN_{tn}/CN_{ngn} group. Specifically, the MCI_{tn}/MCI_{ngn} group exhibited a mean MMSE score that was 1.97 points lower at baseline and decreased by an additional 0.80 points per year relative to the CN_{tn}/CN_{ngn} group. The cognitive trajectory of the CN_{tn}/MCI_{ngn} group was also significantly different from that of the CN_{tn}/CN_{ngn} group, with a mean MMSE score that was 0.72 points lower at baseline and an annual decline that exceeded by 0.32 points that of the CN_{tn}/CN_{ngn} group (Figure 3A; Table S5).

Subsequently, we conducted separate LME models of the Aβ+ and Aβ− subsamples. The diagnostic groups exhibited similar tendencies in cognitive trajectories as observed in the complete dataset, although not all comparisons reached statistical significance (Figure 3B,C; Tables S6 and S7).

4 | DISCUSSION

We previously derived Aβ− NGN for a comprehensive neuropsychological battery using GAMLSS.[10] In this study, we evaluated the added value of NGN in detecting cognitive impairment compared to TN. Our results indicate that NGN enhance diagnostic sensitivity, while preliminary analyses do not reveal any overt loss of specificity.

We observed notable discrepancies between TN- and NGN-based cognitive classifications. Specifically, one of every five (19%) participants considered CN under TN was diagnosed with MCI by NGN, while 3% of those diagnosed with MCI under TN were classified as CN by NGN. These reclassifications were supported by patterns of neuropsychological performance and AD biomarker positivity. Participants consistently classified as CN (CN_{tn}/CN_{ngn}) performed best, followed by those TN-diagnosed with MCI but NGN-classified CN (MCI_{tn}/CN_{ngn}); these, in turn, outperformed participants TN-classified CN but NGN-diagnosed with MCI (CN_{tn}/MCI_{ngn}); finally, participants consistently diagnosed with MCI (MCI_{tn}/MCI_{ngn}) showed the poorest neuropsychological performance. Regarding AD biomarkers, the MCI_{tn}/CN_{ngn} group had the lowest proportion of Aβ+ individuals (8.7%), statistically equivalent to that in the CN_{tn}/CN_{ngn} group (22%). In turn, the proportion of Aβ+ participants in the CN_{tn}/MCI_{ngn} group (48%) more closely resembled that in the MCI_{tn}/MCI_{ngn} group (65%).

These findings suggest that CN_{tn}/MCI_{ngn} individuals likely represent false-negative diagnostic errors under TN. The enhanced sensitivity achieved by NGN was primarily driven by impairments in the FCSRT subtests, detected by NGN but not by TN. This aligns with established evidence that episodic memory deficits are the hallmark of MCI due to AD.[27–29] Conversely, MCI_{tn}/CN_{ngn} individuals might represent false-positive diagnostic errors by TN, possibly related to alternative, more benign etiologies.

Longitudinal analyses further validated the utility of NGN. The CN_{tn}/MCI_{ngn} group demonstrated baseline MMSE scores and annual decline rates that were intermediate between those observed in the CN_{tn}/CN_{ngn} and MCI_{tn}/MCI_{ngn} groups. These trends remained consistent across Aβ+ and Aβ− subgroups, although some comparisons did not reach statistical significance due to limited statistical power.

TABLE 2 Baseline demographic, neuropsychological, and biomarker characteristics across actuarially reclassified diagnostic groups.

Characteristics	CN_{tn}/CN_{ngn} $n = 1066$*	MCI_{tn}/CN_{ngn} $n = 23$*	CN_{tn}/MCI_{ngn} $n = 242$*	MCI_{tn}/MCI_{ngn} $n = 854$*	*p* value†	Effect size‡
Demographics						
Age (years)	63.0 (9.7)	57.4 (11.7)	70.3 (8.8)	68.8 (8.4)	**< 0.001**	$\eta^2 = 0.11$
Education (years)	13.0 (4.7)	12.2 (5.2)	10.0 (4.2)	11.7 (4.8)	**< 0.001**	$\eta^2 = 0.04$
Sex (female)	625 (59%)	14 (61%)	146 (60%)	466 (55%)	0.2	$\varphi c = 0.03$
Clinical measures						
MMSE (/30)	28.5 (1.7)	27.2 (2.8)	26.5 (2.8)	25.2 (3.1)	**< 0.001**	$\eta^2 = 0.28$
Neuropsychological performance (*w*-scores)						
VOSP number location	0.0 (1.0)	−0.5 (1.2)	−0.4 (1.3)	−0.7 (1.5)	**< 0.001**	$\eta^2 = 0.07$
ROCF copy	0.0 (1.0)	−0.4 (1.3)	−0.3 (1.4)	−1.0 (1.8)	**< 0.001**	$\eta^2 = 0.1$
FCSRT total free recall	0.0 (1.0)	−0.9 (0.9)	−1.2 (0.9)	−2.5 (1.0)	**< 0.001**	$\eta^2 = 0.59$
FCSRT total recall	0.0 (1.0)	−0.7 (1.1)	−2.0 (1.5)	−4.9 (2.5)	**< 0.001**	$\eta^2 = 0.62$
FCSRT delayed free recall	0.0 (1.0)	−0.6 (1.0)	−1.4 (1.0)	−2.5 (1.0)	**< 0.001**	$\eta^2 = 0.58$
FCSRT delayed total recall	0.0 (1.0)	−0.5 (1.0)	−2.1 (1.7)	−5.4 (3.0)	**< 0.001**	$\eta^2 = 0.59$
ROCF delayed recall	0.0 (1.0)	−0.4 (1.2)	−0.7 (1.0)	−1.3 (1.0)	**< 0.001**	$\eta^2 = 0.25$
TMT-A	0.0 (1.0)	−0.9 (1.4)	−1.0 (1.8)	−1.6 (2.1)	**< 0.001**	$\eta^2 = 0.18$
TMT-B	0.0 (1.0)	−0.8 (1.0)	−1.0 (1.2)	−1.5 (1.4)	**< 0.001**	$\eta^2 = 0.27$
Digit Span Forward	0.0 (1.0)	−1.0 (0.9)	−0.3 (0.9)	−0.5 (1.0)	**< 0.001**	$\eta^2 = 0.05$
Digit Span Backward	0.0 (1.0)	−1.1 (0.8)	−0.4 (0.9)	−0.6 (1.0)	**< 0.001**	$\eta^2 = 0.09$
Phonetic fluency	0.0 (1.0)	−0.9 (1.0)	−0.6 (0.8)	−1.0 (1.0)	**< 0.001**	$\eta^2 = 0.18$
Semantic fluency	0.0 (1.0)	−0.6 (1.1)	−0.7 (0.7)	−1.1 (0.9)	**< 0.001**	$\eta^2 = 0.25$
BNT	0.0 (1.0)	−0.7 (0.8)	−1.1 (1.5)	−1.9 (2.1)	**< 0.001**	$\eta^2 = 0.24$
CSF Aβ status						
Aβ+	234 (22%)	2 (8.7%)	115 (48%)	558 (65%)	**< 0.001**	$\varphi c = 0.42$

Note: Descriptive and inferential statistics on baseline demographics, MMSE scores, neuropsychological performance, and frequencies of CSF Aβ+ participants across actuarially reclassified diagnostic groups. Raw neuropsychological scores were converted to demographically adjusted *w*-scores (reference group: CN_{tn}/CN_{ngn}) to account for the potential influence of demographic variables on neuropsychological performance and to facilitate comparison across measures. Bold *p* values indicate statistical significance (< 0.05).

Abbreviations: Aβ, amyloid β; Aβ+, Aβ–positive; AD, Alzheimer's disease; ANOVA, analysis of variance; BNT, Boston Naming Test; CN, cognitively normal; CSF, cerebrospinal fluid; FCSRT, Free and Cued Selective Reminding Test; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; ngn, next-generation norms; ROCF, Rey–Osterrieth Complex Figure; SD, standard deviation; TMT-A/B, Trail Making Test Part A/Part B; tn, traditional norms; VOSP, Visual Object and Space Perception Battery.

*Mean (SD); *n* (%).

†One-way ANOVA; Welch's ANOVA; Pearson's chi-squared test.

‡η^2 = eta-squared, φc = Cramér's V.

These results support the utility of NGN in detecting early cognitive impairment and reflect the accelerating nature of cognitive decline observed in previous longitudinal studies.[30] Additionally, consistent with the progressive nature of AD, the Aβ+ CN_{tn}/CN_{ngn} subgroup exhibited a steeper cognitive decline than the Aβ– CN_{tn}/CN_{ngn} subgroup, which was likely composed primarily of individuals without neurodegenerative pathology.

The diagnostic advantage of NGN over TN likely stems from a combination of factors. First, our approach of excluding individuals with biomarker evidence of Aβ accumulation from the normative sample is endorsed by prior research,[31–34] and was further validated by our previous findings.[10] This approach may thus have reduced the risk of attributing to normal aging the cognitive decline due to undetected pathology, thereby enabling a more accurate assessment of neuropsychological performance and AD-related cognitive decline.[35] Second, the GAMLSS framework enables the inclusion of continuous predictors (e.g., age and education) and modeling of all distribution parameters (not just the mean), leading to more precise and realistic normative estimates.[36,37] Finally, adjusting NGN for sex, along with age and education, has likely contributed to their improved accuracy.[38]

These results have relevant implications for clinical practice and research. Current diagnostic criteria and expert consensus recommend that, in clinical settings, AD biomarkers be reserved for cognitively impaired individuals.[8,9] Our findings suggest that NGN could support clinical decision-making by enhancing the identification of individuals who qualify for biomarker testing. Additionally, because

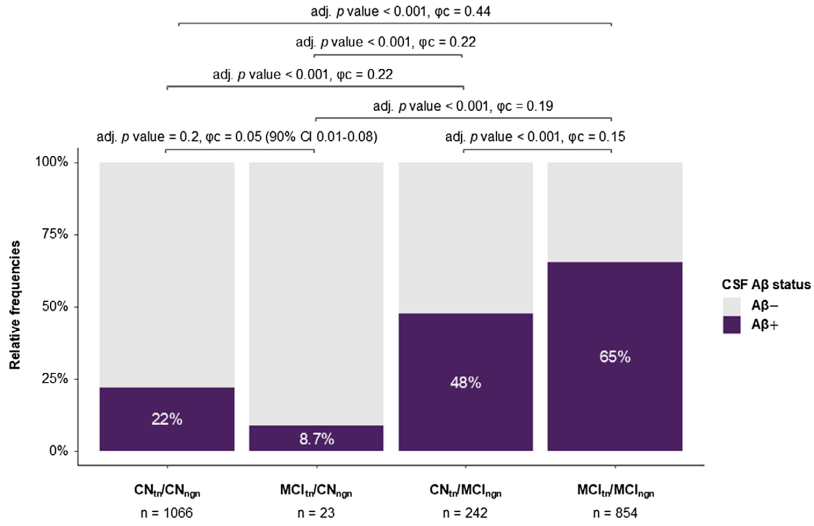


FIGURE 2 Proportions of CSF Aβ+ participants across actuarially reclassified diagnostic groups and Bonferroni-corrected pairwise comparisons. Aβ, amyloid β; Aβ−, Aβ-negative; Aβ+, Aβ-positive; adj. *p* value, Bonferroni-adjusted *p* value; CI, confidence interval; CN, cognitively normal; CSF, cerebrospinal fluid; MCI, mild cognitive impairment; ngn, next-generation norms; tn, traditional norms; φc, Cramér's V.

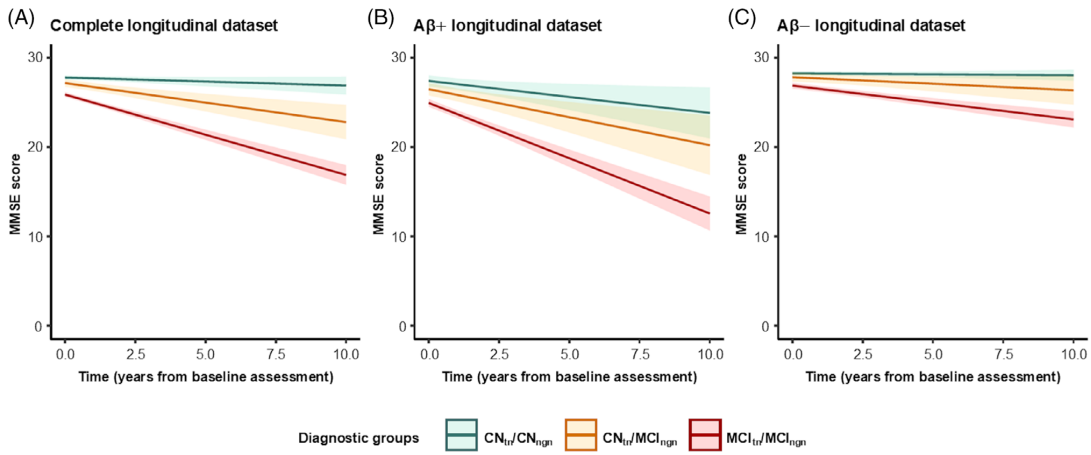


FIGURE 3 Longitudinal trajectories of MMSE scores across actuarially reclassified diagnostic groups. LME model estimates of temporal changes in MMSE scores are shown for the complete longitudinal dataset (A), and for the Aβ+ (B) and Aβ− (C) subsamples. The MCI_{tn}/CN_{ngn} group was not included in the models due to insufficient sample size for reliable analysis. The shaded areas represent 95% CI. Aβ, amyloid β; Aβ−, Aβ-negative; Aβ+, Aβ-positive; CI, confidence interval; CN, cognitively normal; LME, linear mixed-effects; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; ngn, next-generation norms; tn, traditional norms.

copathology occurs frequently, it is recommended that a positive biomarker result should not lead to a diagnosis of AD unless backed by a suggestive clinical phenotype, including a consistent profile of cognitive impairment.[6,8,9,39] In this regard, a comprehensive neuropsychological evaluation covering the main cognitive domains (i.e., memory, language, attention/executive, and visuospatial functions) is essential to capture the nature and extent of the deficits.[40] NGN contribute to the characterization of cognitive impairment and the assessment of severity across these primary domains, thereby informing the interpretation of biomarkers in routine clinical practice. Importantly, timely and accurate detection of AD-related cognitive impairment enables earlier treatment, which may improve patient outcomes and boost the ability of clinical trials to detect drug efficacy.[7,41]

This study has limitations. The first limitation is that the validation sample is not completely independent of the normative dataset. The preceding effort to maximize the sample size of the normative dataset made it unfeasible to obtain a contemporaneous, entirely independent validation sample. However, more than two thirds of the participants (1507 out of 2185, 69%) involved in assessing the utility of NGN were independent of the normative sample. We judged that maintaining the overlapping normative participants in the validation sample (678 out of 2185, 31%) was the best available option, as excluding them would likely have introduced other limitations. Notably, this would have biased the Aβ+ prevalence in the validation sample, particularly in the CN_{tn}/CN_{ngn} group, by enriching it with Aβ+ participants above the expected prevalence in the general population.[42] This overrepresentation of Aβ+ CN_{tn}/CN_{ngn} individuals could dilute the comparative results. Thus, although the lack of entirely independent samples is a limitation, we believe our approach offers the most reliable validation given the constraints. Second, the absence of autopsy or biomarker confirmation for non-AD neurodegenerative diseases limits the interpretability of our findings. To address this limitation, we used longitudinal analyses to capture cognitive deterioration over time as a surrogate marker of neurodegeneration. Finally, because the MCI_{tn}/CN_{ngn} subgroup—providing the most direct test of specificity—was small, and only a minority had at least 1-year follow-up data, our study cannot definitively quantify specificity. Future research should aim to replicate these findings in an independent sample. In addition, future studies could incorporate base-rate approaches that explicitly model false-positive rates[43] and longitudinal clinical outcomes to provide a more rigorous estimate of specificity.

In conclusion, our findings indicate that, by integrating methodological strengths, NGN improve the detection of cognitive impairment, particularly AD-related cognitive impairment. Implementing NGN in clinical and research settings could refine decision-making, enhance clinical trial design, and ultimately improve patient outcomes.

ACKNOWLEDGMENTS

We want to express our gratitude to all participants of this study and all the clinical and research teams involved in their assessment. Alexandre Bejanin acknowledges support from Instituto de Salud Carlos III (ISCIII) and co-funded by the European Union through the Miguel Servet grant (CP20/00038), and the Alzheimer's Association (AARG-22-923680). Mircea Balasa reported receiving support from ISCIII (PI22/00343). Javier Arranz was supported by ISCIII through the Río Hortega Fellowship CM21/00243 and co-funded by the European Union. Nuole Zhu was supported by contract CM21/00113 from ISCIII, co-funded by the European Union. Juan Fortea reports grants from Fundación Tatiana Pérez de Guzmán el Bueno (IIBSP-DOW-2020-151). He was also supported by Horizon2020–Research and Innovation Framework Programme from the European Union (H2020-SC1-BHC-2018-2020). Raquel Sánchez-Valle reported receiving funding from ISCIII (20/00448). Pablo Martínez-Lage reported receiving funding from the Department of Economic Promotion of the Provincial Government of Gipuzkoa (124/16), the Department of Health of the Basque Government (2016111096, S-PR12CH001, S-PR13ZH001), and ISCIII (PI12/02262, PI15/00919). Ignacio Illán-Gala was supported by the Juan Rodés Contract (JR20/0018) from ISCIII, partly funded by the European Social Fund. He is a senior Atlantic Fellow for Equity in Brain Health at the Global Brain Health Institute (GBHI) and receives funding from the Alzheimer's Association (AACSF-21-850193), and the Alzheimer Society (GBHI ALZ UK-21-72097). The SPIN cohort received funding from Fondo de Investigaciones Sanitario, ISCIII (PI13/01532, PI14/01126, PI16/01825, PI17/01019, PI17/01896, PI18/00335, PI18/00435, PI19/00882, PI20/01473, PI20/00836, PI21/01395, PI21/00063, PI21/00791, PI22/00611, PI22/00307, PI23/01786, PI24/00598, PI24/00968, PI24/01087, INT19/00016, INT21/00073, INT23/00048, AC19/00103, DTS22/00111) and the CIBERNED program (Program 1, Alzheimer Disease), jointly funded by Fondo Europeo de Desarrollo Regional, Unioń Europea, "Una manera de hacer Europa". The SPIN cohort was also supported by the National Institutes of Health grants (1R01AG056850-01A1, R21AG056974, R01AG061566, 1R01AG081394-01, 1R61AG066543-01, and R01AG080470), by Generalitat de Catalunya (2017-SGR-547, 2021-SGR-00979, SLT006/17/125, SLT006/17/119, SLT002/16/408), Fundació La Marató de TV3 (20141210, 044412, and 20142610), Ajuntament de Barcelona in collaboration with Fundació 'la Caixa' (23S06157-001), Fundació Catalana Síndrome de Down, and Fundació Víctor Grífols i Lucas. Horizon 21 Consortium is partly funded by Jérôme Lejeune Foundation. The GAP study was supported by CITA-Alzheimer Foundation, ISCIII (PI12/02262 and PI1500919), and the Department of Health of the Basque Government (S-PR12CH001 and S-PR13ZH001). The DEBA project was partially funded by the Department of Economic Promotion of the Provincial Government of Gipuzkoa (2016 Support Programme for the Gipuzkoa Network for Science, Technology, and Innovation) and the Department of Health of the Basque Government (2016111096).

CONFLICT OF INTEREST STATEMENT

Sara Rubio-Guerra reported honoraria for educational events from Esteve Pharmaceuticals. María Belén Sánchez-Saudinós reported receiving travel support for attending meetings from Lilly and serving on an advisory board for Novo Nordisk. Miren Altuna reported receiving honoraria for educational events from Lilly, Esteve, ITAMAR, Almirall, and KERN Pharma; as well as support for attending meetings or travel from Lilly, Esteve, and Almirall. Javier Arranz reported receiving honoraria for educational events from Roche, Esteve, and Lilly, and support for attending meetings or travel from Esteve Pharmaceuticals, S.A. Daniel Alcolea reported receiving consulting fees from Grifols S.A., Lilly, Fujirebio-Europe, and Roche Diagnostics; honoraria for educational events from Fujirebio-Europe, Roche Diagnostics, Nutricia, Krka Farmacéutica S.L., Zambon S.A.U., Esteve Pharmaceuticals, S.A., Neuraxpharm, Alter, and Lilly; and support for attending meetings or travel from Fujirebio-Europe, Lilly, and Nutricia. Daniel Alcolea also participated on advisory boards for Grifols S.A., Lilly, Fujirebio-Europe, and Roche Diagnostics. Alberto Lleó reported receiving consulting fees from Eisai, Esteve, Fujirebio-Europe, Roche, Grifols S.A., and Lilly. Juan Fortea reported receiving consulting fees from Lundbeck, Ionis, and AC Immune; honoraria for educational events from Roche, Esteve,

Biogen, Laboratorios Carnot, Adamed, Life Molecular Imaging, Eisai, and Lilly. Juan Fortea has participated on advisory boards for AC Immune, Alzheon, Zambon, Lilly, Roche, Eisai, and Perha. Juan Fortea has held leadership or fiduciary roles in the Spanish Neurological Society, T21 Research Society, Lumind Foundation, Jérôme-Lejeune Foundation, Alzheimer's Association, Health Research Board, and Dementia Trials Ireland (all without payments). Juan Fortea also participated in Study Sections for the European Commission, National Institutes of Health, and Instituto de Salud Carlos III, with payments received for these activities. Juan Fortea reports receipt of equipment, materials, or other services from Life Molecular Imaging, provided to his institution. Daniel Alcolea, Alberto Lleó, and Juan Fortea declare a filed patent application (WO2019175379 A1 Markers of synaptopathy in neurodegenerative disease). Raquel Sánchez-Valle reported receiving consulting fees from Lilly (paid to her institution) and UCB (paid personally). Pablo Martínez-Lage reported receiving consulting fees from Lilly, Roche, and Eisai; honoraria for educational events from Lilly, Roche, Eisai, Nutricia, and Almirall; and support for attending meetings or travel from Lilly, Roche, Eisai, and Nutricia. Ignacio Illán-Gala reported receiving honoraria for educational events from Esteve, Sociedad Española de Neurología, Societat Catalana de Neurologia, Lilly, Kern Pharma, and Almirall; support for attending meetings or travel from Esteve and Almirall; and participation on advisory boards for UCB and Nutricia. The remaining authors declare no conflicts of interest. Author disclosures are available in the Supporting Information.

CONSENT STATEMENT

Written informed consent was obtained from all human subjects before enrollment in the study.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Rubio-Guerra S, Sala I, Sánchez-Saudinós MB, et al. Amyloid-negative neuropsychological norms: Added value in the era of biomarkers and disease-modifying therapies. *Alzheimer's Dement*. 2025;17:e70223. https://doi.org/10.1002/dad2.70223

5.3. Article 3: A novel *CHMP2B* splicing variant in atypical presentation of familial frontotemporal lobar degeneration

Sara Rubio-Guerra, Sara Bernal, David Almenta, Josefina Pérez-Blanco, Valle Camacho, Isabel Sala, Mª Belén Sánchez-Saudinós, Jesús García Castro, Judit Selma-González, Miguel Ángel Santos-Santos, Álvaro Carbayo, Janina Turon-Sans, Ricard Rojas-Garcia, Daniel Alcolea, Juan Fortea, Alberto Lleó, Oriol Dols-Icardo, Ignacio Illán-Gala. A Novel *CHMP2B* Splicing Variant in Atypical Presentation of Familial Frontotemporal Lobar Degeneration. Ann Clin Transl Neurol. 2025 Apr 17;acn3.70023. https://doi.org/10.1002/acn3.70023

Annals of Clinical and Translational Neurology

BRIEF COMMUNICATION OPEN ACCESS

A Novel *CHMP2B* Splicing Variant in Atypical Presentation of Familial Frontotemporal Lobar Degeneration

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Received: 17 December 2024 | **Revised:** 27 January 2025 | **Accepted:** 12 February 2025

Funding: This work was supported by Global Brain Health Institute (GBHI ALZ UK-21-720973), Departament de Salut, Generalitat de Catalunya (SLT006/17/125), Fundación BBVA, Fondation Jérôme Lejeune (#202307 and PDC-2023-51), Fundación Española para el Fomento de la Investigación de la Esclerosis Lateral Amiotrófica, Instituto de Salud Carlos III (CM21/00057, CM23/00176, INT19/00016, INT23/00048, JR20/00018, Juan Rodés contract, PI18/00435, PI21/00791, PI21/01395, PI22/00611, PI23/00845, and PI24/01087), Alzheimer's Association (AACSF-21-850193, AACSF-22-972945, and AARF-22-924456), BrightFocus Foundation, Horizon 2020 Framework Programme, National Institutes for Health (R01AG080470), and Fundación HNA, https://www.fundacionhna.es/conocenos and Fundació Tatiana Pérez de Guzmán el Bueno.

ABSTRACT

C-truncating variants in the charged multivesicular body protein 2B (*CHMP2B*) gene are a rare cause of frontotemporal lobar degeneration (FTLD), previously identified only in Denmark, Belgium, and China. We report a novel *CHMP2B* splice-site variant (c.35-1G>A) associated with familial FTLD in Spain. The cases were two monozygotic male twins who presented at ages 62 and 66 years with a slowly progressive behavioral variant of frontotemporal dementia and a syndrome mimicking dementia with Lewy bodies, respectively. Functional and in silico analyses supported the pathogenicity of this variant. Our findings contribute new insights into the genetic landscape and clinical heterogeneity of FTLD.

1 | Introduction

Frontotemporal lobar degeneration (FTLD) is a pathological umbrella term for a heterogeneous group of neurodegenerative diseases primarily affecting the frontal and temporal lobes of the brain [1]. Clinically, FTLD can present with behavioral changes, speech and language impairment, and pyramidal or extrapyramidal motor dysfunction [1]. Pathologically, FTLD is classified into four main molecular subtypes according to the biochemical composition of intracellular protein inclusions, namely: transactive response DNA-binding protein 43 (FTLD-TDP, which accounts for about 45% of FTLD cases), microtubule-associated protein tau (FTLD-tau, 45%), fused in sarcoma (FTLD-FUS, 9%), and ubiquitin–proteasome system positive inclusions that are negative for TDP-43, tau, and FUS (FTLD-UPS, 1%) [2, 3].

FTLD clinical-pathological correlations are imperfect; however, approximately 20%–25% of cases are caused by autosomal

Oriol Dols-Icardo and Ignacio Illán-Gala contributed equally to the seniorship of this work.

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dominant variants with strong genetic-pathological associations. The most common FTLD-causing genes are *C9orf72*, *GRN* (both leading to FTLD-TDP), and *MAPT* (linked to FTLD-tau), but other less commonly reported genetic causes have been described [1]. C-truncating variants in the charged multivesicular body protein 2B (*CHMP2B*) gene, associated with FTLD-UPS, have been previously documented only in Denmark [4], Belgium [5], and China [6].

Here, we report a novel splicing variant in the first *CHMP2B*-associated FTLD family identified in Spain. We provide compelling evidence supporting the pathogenicity of this variant and describe its atypical phenotypic expression in two monozygotic (identical) twins.

2 | Case Reports

2.1 | Family History

Family history was notable for several neuropsychiatric cases of unknown etiology, including the suicide death of 2 second-degree relatives. The patients' father had died at age 86 from late-onset dementia. Their mother had died from a stroke at age 61. The monozygotic twin patients were the third and fourth of five siblings, the rest of whom suffered from no neurological or psychiatric problems. The proband had one healthy daughter. Family history was therefore compatible with an autosomal dominant inheritance pattern (Figure 1).

2.2 | Case 1

The index case was a right-handed male with 20 years of formal education. He presented at age 64, at his wife's initiative, with a 2-year history of slowly progressive personality change characterized by compulsive behaviors (e.g., object and animal hoarding), diminished empathy (e.g., social and emotional detachment), and changes in his dietary and eating behavior (e.g., idiosyncratic food preferences, taking food from others' plates). She also reported difficulties in attention and language. The patient exhibited limited awareness of these changes.

At presentation, his Mini-Mental State Examination (MMSE) [7] score was 28/30, and his Clinical Dementia Rating Scale-Sum of Boxes (CDR-SoB) [8] score was 3.5. Neuropsychological assessment revealed executive dysfunction and language impairment characterized by deficits in naming, verbal fluency, and comprehension, with preserved repetition and reading. Episodic memory and visuospatial skills were also relatively preserved. He exhibited no motor neuron signs or other abnormalities on neurological examination. Routine laboratory work-up was unremarkable. Brain magnetic resonance imaging (MRI) and [^{18}F]fluodeoxiglucose positron emission tomography-computed tomography ([^{18}F]FDG-PET/CT) showed mild global atrophy and normal metabolism (Figure 2). Cerebrospinal fluid (CSF) biomarker analysis [amyloid β 1–42 (Aβ42), phosphorylated tau at threonine-181 (p-tau), total tau (t-tau), and Aβ42/t-tau ratio] ruled out Alzheimer's disease pathophysiology as the underlying etiology. Genetic testing for the *C9orf72* repeat expansion was negative.

Based on clinical and complementary test information, a diagnosis of a behavioral variant of frontotemporal dementia (bvFTD) was initially considered [10]. However, the observed slow rate of cognitive and functional decline during the first 4 years of follow-up led the clinical team to withhold a definitive diagnosis of frontotemporal dementia. He was referred for evaluation by an experienced psychiatrist, who determined that a personality disorder was a potential explanation for the patient's symptoms. All of the above led to considering the possibility of a bvFTD phenocopy syndrome [11]. Nonetheless, after 6 years of follow-up, the patient's MMSE score had declined to 25/30, and

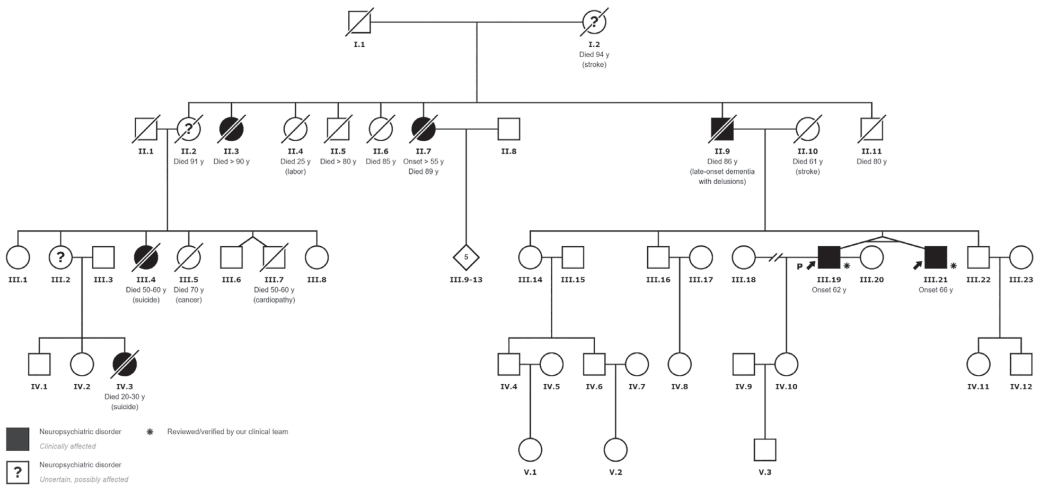


FIGURE 1 | Pedigree chart. The arrows mark the cases reported in the main text. Letter P indicates the proband (case 1). Years (y) are the ages at onset or death. Death cause is given in parentheses when known. Detailed clinical data could not be obtained for all affected family members due to limited informant recall and their sparse contact with second-degree family members living in another city. The assessment of these relatives by our clinical team was not possible either because they had already deceased at the time of our patients' evaluation.

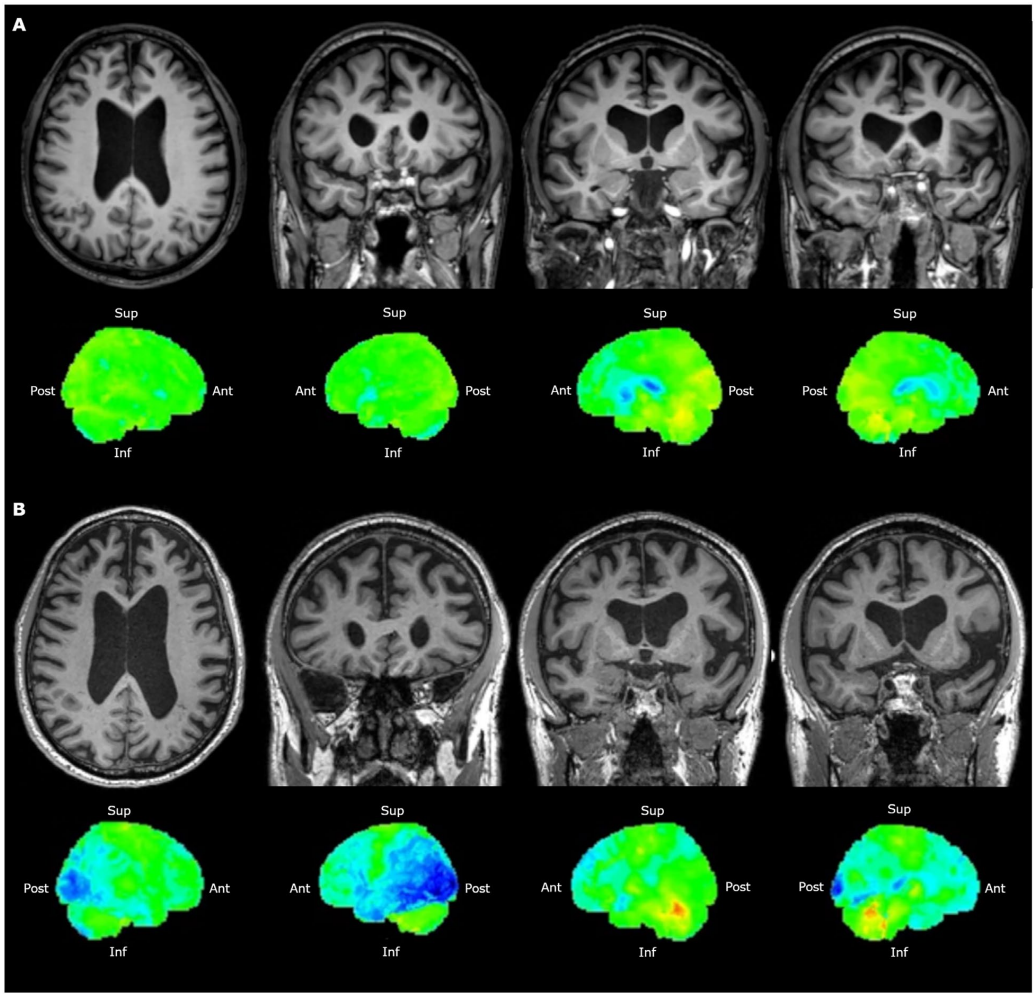


FIGURE 2 | Neuroimaging findings. Neuroimaging findings in Cases 1 (Panel A) and 2 (Panel B). The upper row in each panel displays T1-weighted magnetic resonance imaging (MRI), left to right: axial, and coronal slices showing anterior cingulate and orbitofrontal, frontal insula, and anterior temporal regions [9]. All MRI images are in radiological orientation. The bottom row in each panel displays 3-dimensional stereotactic surface projection (3D-SSP) analyses of [^{18}F]fluodeoxiglucose positron emission tomography-computed tomography ([^{18}F]FDG-PET/CT) images, left to right: right lateral, left lateral, right medial, and left medial. Orientation in the body is described as anterior (Ant), posterior (Post), superior (Sup), and inferior (Inf). The intensity normalization is based on the cerebellum as a reference region. Green colors indicate normal glucose metabolism, blue colors indicate hypometabolism, and orange-red colors indicate high metabolic activity. (A) Case 1 showed mild global atrophy and normal metabolism of the brain. (B) Case 2 exhibited prominent, left-lateralized, atrophy and hypometabolism of occipitoparietotemporal and frontal regions, with preservation of the posterior cingulate cortex.

his CDR-SoB score had reached 4. The gradual but persistent cognitive and functional deterioration, in conjunction with progressive atrophy evident on longitudinal MRI assessments quantified through visual atrophy scales [9] ultimately supported a diagnosis of a slowly progressive neurodegenerative disease.

2.3 | Case 2

His monozygotic male twin, a right-handed individual with 12 years of formal education, was unmarried and living alone. He presented at age 66 with language impairment and delusional beliefs about his neighbor poisoning him with chemical substances.

At the first visit, his MMSE score was 16/30, and neuropsychological testing revealed widespread cognitive impairment consistent with mild dementia. He soon developed dyscalculia, speech stereotypies, hyperorality, and motor impairment characterized by right-dominant akinetic-rigid syndrome. He exhibited no motor neuron signs. Blood analyses were normal. Brain MRI and [^{18}F]FDG-PET/CT imaging revealed

left-predominant occipitoparietotemporal and frontal hypometabolism, with preservation of the posterior cingulate cortex (Figure 2). CSF biomarkers [Aβ42, amyloid β 1–40 (Aβ40), p-tau, t-tau, and Aβ42/Aβ40 ratio] ruled out Alzheimer's disease pathophysiology.

Based on the clinical presentation and imaging findings, including parkinsonism, psychotic symptoms (interpreted as probable visual hallucinations), and posterior brain changes with a cingulate island sign, he was initially diagnosed with dementia with Lewy bodies [12] and was prescribed donepezil 10 mg per day. After 4 years of follow-up, he was dependent for basic activities of daily living, and his MMSE score had declined to 6/30.

2.4 | Genetic Analysis

Diagnostic uncertainty and the emergence of additional neuropsychiatric cases in the family prompted a deeper genomic study of the index case. Whole exome sequencing revealed that the proband harbored a novel heterozygous *CHMP2B* variant in the canonical splice acceptor site of intron 1 (NM_014043.4:c.35-1G>A), which was confirmed by Sanger sequencing. Importantly, disease-causing variants were ruled out in other genes related to FTLD, amyotrophic lateral sclerosis, Alzheimer's disease, and dementia with Lewy bodies (Table 1). The G-to-A change caused a shift in the reading frame that resulted in a premature stop codon, predicting a C-truncated protein composed of only the first 16 amino acids (p.Asp12Glyfs*5). The variant had not been reported in the most comprehensive population database (gnomAD v4.1.0) and was strongly predicted to be deleterious by two deep learning models, spliceAI (score = 0.99) [13] and Pangolin (score = 1) [14]. The variant was subsequently identified by Sanger sequencing in his affected brother. Written informed consent was obtained from both patients.

To confirm the variant's pathogenicity, peripheral blood RNA of both siblings was reverse-transcribed and amplified using polymerase chain reaction. Sanger sequencing of the cDNA identified a wild-type transcript and a variant transcript skipping exon 2 that was not found in RNA samples from non-variant carriers (Figure 3). The detection of the aberrant transcript confirmed that the variant altered the normal splicing of *CHMP2B* and led to classifying the variant as pathogenic according to the ACMG guidelines (PVS1 + PM1 + PS3) [15].

TABLE 1 | Studied genes.

ABCA1	*ABCA7*	*ADAM10*	*ADH1C*	*ALS2*
ANG	*ANXA11*	*APP*	*ARHGEF28*	*ARPP21*
ATP13A2	*ATP1A3*	*ATP6AP2*	*ATP8B4*	*ATXN2*
C19orf12	*C21orf2* (=*CFAP410*)	*C9orf72*	*CCNF*	*CHCHD10*
CHCHD2	*CHMP2B*	*CP*	*CTSF*	*CYLD*
DAO	*DCTN1*	*DJ1* (=*PARK7*)	*DNAJB2*	*DNAJC12*
DNAJC6	*EIF4G1*	*ERBB4*	*EWSR1*	*FBXO7*
FIG4	*FTL*	*FUS*	*GBA*	*GCH1*
GIGYF2	*GLB1*	*GLE1*	*GLUD2*	*GRN*
HNRNPA1	*HNRNPA2B1*	*HTRA2*	*ITM2B*	*KIF5A*
LRRK2	*MAPT*	*MATR3*	*NEFH*	*NEK1*
NPC1	*NPC2*	*OPTN*	*PDGFB*	*PDGFRB*
PFN1	*PINK1*	*PLA2G6*	*POLG*	*PRKN* (=*PARK2*)
PRKRA	*PRNP*	*PRPH*	*PSEN1*	*PSEN2*
PTS	*RAB39B*	*SETX*	*SIGMAR1*	*SLC18A2*
SLC20A2	*SLC30A10*	*SLC30A3*	*SLC39A14*	*SLC6A3*
SMPD1	*SNCA*	*SNCAIP*	*SNCB*	*SOD1*
SORL1	*SPG11*	*SPR*	*SQSTM1*	*SS18L1*
SYNJ1	*TAF1*	*TAF15*	*TARDBP*	*TBK1*
TBP	*TH*	*TIA1*	*TREM2*	*TRPM7*
TUBA4A	*UBQLN2*	*UCHL1*	*UNC13A*	*VAPB*
VCP	*VPS13C*	*VPS35*	*WDR45*	*XPR1*

Note: List of the 110 genes screened in the index case. The selection of genes of interest for analysis was based on previously described associations with dementing neurodegenerative diseases, amyotrophic lateral sclerosis, or parkinsonian syndromes.

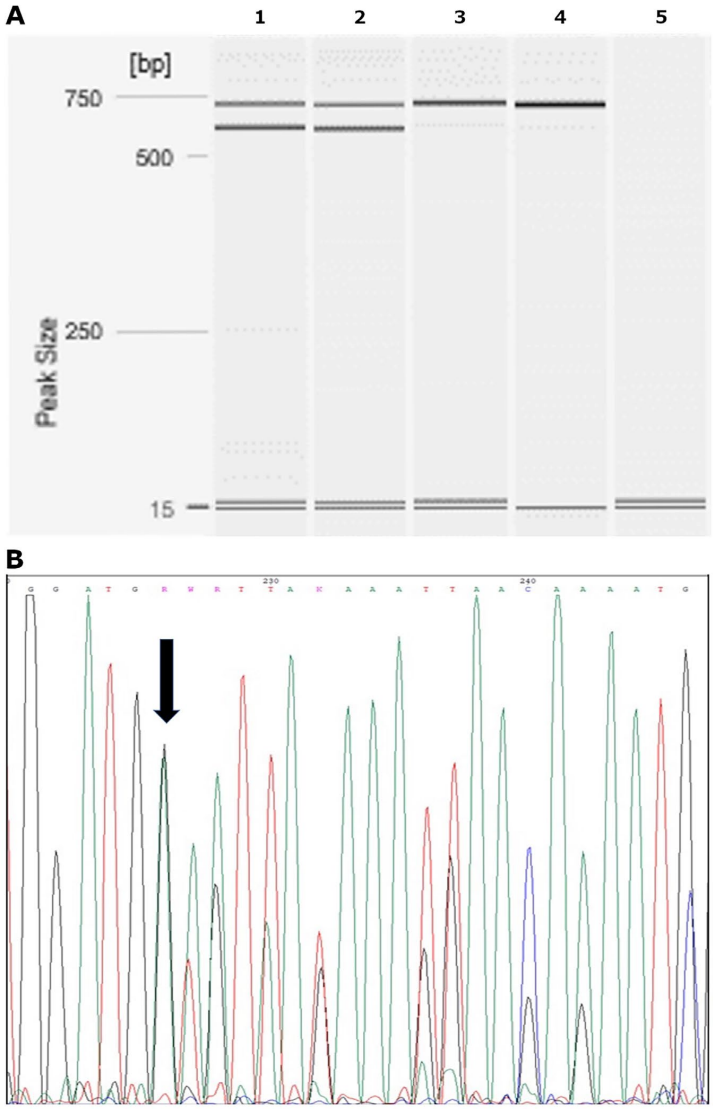


FIGURE 3 | Genetic analysis. Functional RNA study of the genetic variant identified in the *CHMP2B* gene (NM_014043.4:c.35-1G>A). (A) In the reverse-transcription polymerase chain reaction (RT-PCR) image, two bands can be observed in Lanes 1 and 2 (cDNA samples from the two patients carrying the mutation): One band of 750 bp and another, smaller band, of approximately 660 bp. In contrast, a single band appears in Lanes 3 and 4 (samples from non-mutation carriers). Lane 5: Negative control (no template cDNA). (B) Sanger sequencing of cDNA sample from a patient carrying the heterozygous *CHMP2B* variant.

3 | Discussion

This report presents a novel splice-site variant in Spain's first pedigree with *CHMP2B*-associated FTLD. *CHMP2B* (chromosome 3) is a 6-exon gene encoding a 213-amino acid protein of the endosomal sorting complex required for transport-III (ESCRT-III). ESCRT-III is involved in the endosomal-lysosomal and autophagy pathways, essential for the lysosomal degradation of endocytosed and cytoplasmic cellular components [16]. Genetic variants in *CHMP2B* are the primary known cause of FTLD-UPS, a neuropathological subtype characterized by hippocampal and frontal neuronal cytoplasmic inclusions that stain for markers of the ubiquitin-proteasome system, such as ubiquitin and p62, but are negative for TDP-43, tau, and FUS [2]. Neuronal accumulation of aberrant endosomes and autophagic organelles has been

observed in *CHMP2B* variant carriers and other neurodegenerative diseases, presumably reflecting the impairment in endosomal-lysosomal trafficking [2, 16–18].

While multiple *CHMP2B* variants have been identified, only those loss-of-function variants resulting in C-terminal truncations of the CHMP2B protein are considered pathogenic. In contrast, the pathogenicity of missense variants remains unclear [6, 16]. C-truncating variants in *CHMP2B* have been previously identified only in Denmark [4], Belgium [5], and China [6]. The variants found in both a Danish FTLD pedigree (c.532-1G>C) and a Chinese patient (c.532-2A>T) occurred in the splice acceptor site of intron 5. They were predicted to translate into proteins lacking the final wild-type 36 amino acids. In a Belgian familial FTLD patient, a nonsense variant was identified in exon 5 (c.493C>T), predicting the deletion of the final 49 amino acids of the protein. Here, we report two affected monozygotic twins harboring a heterozygous variant in the splice acceptor site of intron 1 (c.35-1G>A). We demonstrate that the splicing variant reported impacts RNA processing, resulting in a truncated mRNA. However, functional studies are needed to confirm its effects at the protein level. The variant reported herein, as previously shown for similar variants in *CHMP2B*, may result in a truncated protein, impairing endosomal function through a gain-of-function mechanism [5]. On the other hand, it may result in the loss of the entire protein through nonsense-mediated decay. In this sense, the depletion of CHMP2B has been demonstrated to alter dendritic branching and induce synaptic defects [19], thus supporting the notion that this variant is pathogenic through a loss-of-function mechanism. Functional studies will be needed to determine whether the splicing variant is pathogenic through a loss-of-function or gain-of-function mechanism.

The presentation of the index case with a slowly progressive personality change aligns with previous literature identifying bvFTD as the most frequent phenotype among *CHMP2B* variant carriers. Psychosis, dyscalculia, and progressive aphasia have also been documented early in the disease course, as was observed in the second case. Disease progression frequently leads to mutism and extrapyramidal signs. Neuroimaging in *CHMP2B*-related FTLD typically shows mild global atrophy at diagnosis, with some cases exhibiting prominent parietal involvement. The usual age of onset is between 46 and 70 years, and disease duration ranges from 3 to more than 20 years [6, 16, 20].

The history of suicide in this family warrants attention, given prior studies suggesting a higher prevalence of suicidality among bvFTD patients and FTLD mutation carriers [21–23]. Indeed, a parental history of suicide has been recently reported in one *CHMP2B* variant case [6]. However, the notion of the *CHMP2B* variant being the cause of the two suicide cases in this family remains speculative, as segregation analysis data are not yet available. Equally speculative is whether the phenotypic differences within these two monozygotic twins are explained only by nongenetic (i.e., environmental) factors, or if they could result from different tissue-specific variant effects in the brain. Nevertheless, individuals with a family history of personality change, suicide, or dementia with Lewy bodies may benefit from screening for *CHMP2B* variants. Future studies should better elucidate the prevalence and natural history of *CHMP2B*-associated FTLD.

Taken together, the findings of this report provide valuable insights into the genetic landscape, disease mechanisms, and clinical heterogeneity of FTLD. Our data underscore the importance of comprehensive genetic testing with a low threshold for suspicion, which has important implications for achieving a definitive FTLD diagnosis.

Author Contributions

S.R.-G., O.D.-I., and I.I.-G. conceived and designed the study. S.R.-G., S.B., D.A., J.P.-B., V.C., I.S., M.B.S.-S., J.G.C., J.S.-G., M.Á.S.-S., Á.C., J.T.-S., R.R.-G., D.A., J.F., A.L., O.D.-I., and I.I.-G. carried out the acquisition and analysis of data. S.R.-G., S.B., O.D.-I., and I.I.-G. drafted the manuscript.

Acknowledgments

We thank the patients and family caregivers for their altruism in allowing us to share their cases to benefit the medical community.

I.I.-G. is a senior Atlantic Fellow for Equity in Brain Health at the Global Brain Health Institute (GBHI) and receives funding from the GBHI, the Alzheimer's Association, and the Alzheimer Society (GBHI ALZ UK-21-720973 and AACSF-21-850193). I.I.-G. was also supported by the Juan Rodés Contract (JR20/0018).

This study has been funded by the Instituto de Salud Carlos III (ISCIII) through the projects PI18/00326, PI21/01395 and PI24/01087 to O.D.-I., PI23/00845 to R.R.-G., and PI21/00791 and PI24/00598 to I.I.-G., and co-funded by the European Union. FEDER.

This work was supported by the Alzheimer's Association Clinician Scientist Fellowship Program AACSF-21-850193 awarded to I.I.-G.

O.D.-I. receives funding from the Fundación Española para el Fomento de la Investigación de la Esclerosis Lateral Amiotrófica (FUNDELA—'Por un mundo sin ELA'), the Alzheimer's Association (AARF-22-924456), and the Fondation Jérôme Lejeune (PDC-2023-51; #202307).

J.G.C. is supported by a Río Hortega grant (CM23/00176) from the Carlos III National Institute of Health of Spain, partly funded by the European Social Fund Plus.

This study was supported by the Fondo de Investigaciones Sanitario, Carlos III Health Institute (INT21/00073, PI20/01473 and PI23/01786 to J.F., PI18/00435, PI22/00611, INT19/00016 and INT23/00048 to D.A., PI17/01896, PI20/1330 and AC19/00103 to A.L., and PI19/00882 to M.S.S.) and the Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas Program 1, partly jointly funded by Fondo Europeo de Desarrollo Regional, Unión Europea, Una Manera de Hacer Europa. This work was also supported by the National Institutes of Health grants (R01 AG056850, R21 AG056974, R01 AG061566, R01 AG081394 and R61AG066543 to J.F., and R01AG080470), the Alzheimer's Association clinician scientist fellowship (AACSF-22-972945), ADNI (U01 AG024904), the Department de Salut de la Generalitat de Catalunya, Pla Estratègic de Recerca I Innovació en Salut (SLT006/17/00119 to J.F., and SLT006/17/125 to D.A.). It was also supported by Fundación Tatiana Pérez de Guzmán el Bueno (IIBSP-DOW-2020-151 to J.F.) and the Horizon 2020–Research and Innovation Framework Programme from the European Union (H2020-SC1-BHC-2018-2020 to J.F.).

The family tree was built using TreeStudio v2.0.8242 © (Health in Code S.L.).

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

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

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6. GLOBAL SUMMARY OF THE RESULTS

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This doctoral thesis aims to enhance the timeliness and accuracy of diagnosis of neurodegenerative diseases presenting with cognitive and/or behavioral impairment by refining neuropsychological norms and the use of genetic testing.

To address this aim, this thesis comprises three studies employing two complementary approaches. On the one hand, the first two studies developed and validated refined neuropsychological norms grounded in a large multicenter cohort, allowing for statistical generalizability of the results. On the other hand, the third study focused on the unique features of two single cases to explore the potential of genetic testing to improve the diagnosis of FTLD; although grounded on specific instances, the findings of the case study illustrate theoretical generalizability to other similar cases.

The following paragraphs summarize the aims, designs, and key results of these three individual studies, each contributing to the overall purpose of the thesis.

The first study represents a pioneering effort to develop AD-negative neuropsychological norms using the GAMLSS framework, adjusting for age, education, and sex as appropriate. To this end, we selected a multicenter sample of 774 Aβ− CN adults aged 30 to 90 years. Norm-based z-scores were estimated for a comprehensive neuropsychological battery of 14 tests covering the main cognitive domains (i.e., memory, language, attention/executive, and visuospatial functions). For each neuropsychological measure, the GAMLSS framework enabled continuous, smoothed adjustments of each distribution parameter across age and education. Moreover, each distribution parameter was independently adjusted for sex, allowing the effects of age, education, and sex to vary across the location, scale, and shape components of the model. Of note, in contrast to previous neuropsychological norms, which found no significant influence of sex in any of the tests included in this study, sex had a statistically significant effect on one or more GAMLSS parameters in 7 of the 14 tests. To support the practical implementation of the norms, we developed an open-access, web-based calculator that allows the demographically adjusted

z-scores to be obtained in both tabular and graphical formats (https://cogstats.shinyapps.io/cogstats/; **Table 1** and **Figure 5** present neuropsychological data from a representative clinical case).

Neuropsychological test	Raw score	Scaled score (TN)	z-score (NGN)
VOSP number location	6/10	6	-2.00
ROCF copy	17.5/36	6	-2.30
FCSRT total free recall	13/48	8	-1.90
FCSRT total recall	31/48	8	-1.40
FCSRT delayed free recall	2/16	7	-2.00
FCSRT delayed total recall	8/16	7	-2.10
ROCF delayed recall	5.5/36	7	-1.20
TMT-A	59 s	10	-0.30
TMT-B	127 s	10	-0.10
Phonetic fluency	9 words	9	-0.90
Semantic fluency	17 words	10	0.30
Boston Naming Test	10/15	8	-1.80

Table 1. A 77-year-old female with 11 years of education presented with a 3-year history of insidiously progressive memory complaints, being independent in activities of daily living. Neurological examination was normal. Her MMSE score at the first visit was 24/30, with errors in temporal orientation (−2), calculation and spelling (−1), and delayed recall (−3). The table shows her performance on formal neuropsychological assessment, including raw scores, as well as normed scores based on both current norms (TN) and the normative data developed in this thesis (NGN). Discrepancies between TN and NGN include poorer performance on NGN-based scores on almost all measures, but most notably on memory measures. Brain MRI showed no evidence of vascular contribution or focal atrophy. Laboratory work-up was normal. Plasma p-tau217 concentrations were consistent with AD.

Abbreviations: AD, Alzheimer's disease; FCSRT, Free and Cued Selective Reminding Test; MMSE, Mini-Mental State Examination; MRI, magnetic resonance imaging; NGN, next-generation norms; p-tau217, phosphorylated tau at threonine 217; ROCF, Rey-Osterrieth Complex Figure; TMT-A/B, Trail Making Test Part A/Part B; TN, traditional norms; VOSP, Visual Object and Space Perception Battery.

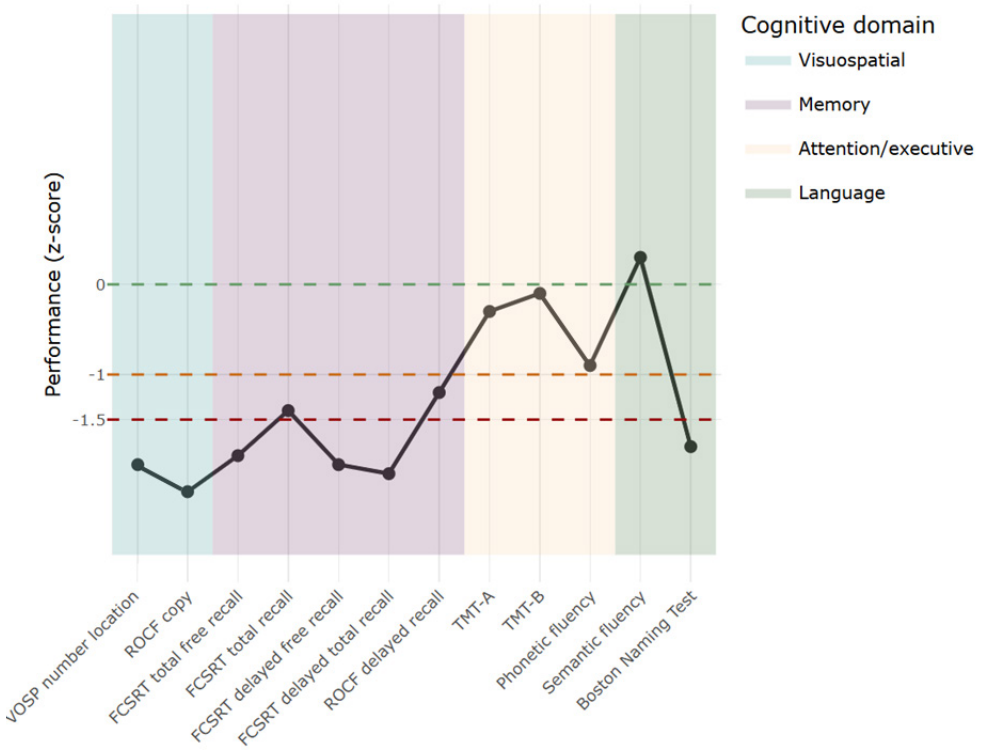


Figure 5. Graphical display of the neuropsychological performance of the same patient presented in Table 1 based on the normative data developed in this thesis (NGN).

Abbreviations: FCSRT, Free and Cued Selective Reminding Test; NGN, next-generation norms; ROCF, Rey-Osterrieth Complex Figure; TMT-A/B, Trail Making Test Part A/Part B; VOSP, Visual Object and Space Perception Battery.

The second, complementary study was intended to evaluate the clinical utility of the norms in detecting the earliest stages of cognitive impairment, compared with current reference data. For this purpose, we applied actuarial neuropsychological criteria to a multicenter cohort of 2405 individuals without dementia (i.e., either CN or MCI): participants were actuarially classified as CN or MCI using both current and our novel norms. Compared to currently used norms, our novel norms yielded tighter associations with neuropsychological performance, proportions of CSF AD biomarker positivity, and longitudinal cognitive outcomes. Specifically, our novel norms identified 19% of MCI cases among individuals classified as CN by current norms, likely representing false-

negative errors with current reference values. By contrast, 3% of individuals diagnosed with MCI under current norms were better classified as CN by our novel norms, likely representing false-positive errors with current norms. Together, these findings suggest that our novel norms streamline the detection of MCI – particularly MCI due to AD.

The third study of this thesis focused on the specificities of the cases of two monozygotic twins, who presented in their 60s with a slowly progressive bvFTD syndrome and a dementia syndrome resembling DLB. Their father had died with late-onset dementia of unknown etiology, and their paternal second-degree relatives had an autosomal dominant family history of neuropsychiatric disorders not otherwise specified, including two suicide cases. Diagnostic uncertainty during long-term follow-up prompted a comprehensive WES study of the bvFTD proband, which revealed a novel heterozygous pathogenic variant in the *CHMP2B* gene. The *CHMP2B* variant was subsequently confirmed by Sanger sequencing in both siblings, thereby finally establishing a definitive diagnosis of the rare FTLD-UPS pathology in both cases. Previously reported only in Denmark, Belgium, and China, this is the first identification of *CHMP2B*-associated FTLD in Spain.

Collectively, the findings establish refined neuropsychological norms and low-threshold comprehensive FTLD genetic testing as tools with considerable potential for enhancing the timeliness and accuracy of diagnosis of neurodegenerative diseases presenting with cognitive and/or behavioral impairment.

7. GLOBAL SUMMARY OF THE DISCUSSION

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Over the course of this doctoral thesis (2022-2026), the field of ADRD has evolved significantly.

In the two years following the 2021 controversial approval of aducanumab, the pivotal phase III clinical trials of lecanemab(202) and donanemab(203) were published, and both anti-Aβ monoclonal antibodies were subsequently approved in many countries, including the European Union. Currently, more than 130 additional drugs are being evaluated in AD clinical trials.(204) In this context, the implementation of blood-based biomarkers in routine clinical practice promises to expand access to AD-targeted therapies.(111,122)

In parallel, there has been steady progress in gene-targeted therapies for FTLD. Following the path of AD therapeutics, tofersen –an antisense oligonucleotide (ASO) treatment for individuals with ALS due to pathogenic variants in the *SOD1* gene (*SOD1*-ALS)(205)– received accelerated approval by the FDA in April 2023 and was subsequently approved by the EMA in 2024. In the meantime, other FTLD gene-targeted therapies continue advancing toward clinical implementation. The approval of tofersen, making it the first gene-targeted treatment for ALS, has prompted the development of foundational consensus guidelines on genetic testing for individuals with ALS,(206) while genetic testing guidelines for non-ALS FTLD patients have yet to be established.(198,207)

These advances emphasize the importance of early and accurate diagnosis of ADRD. The three studies included in this thesis align seamlessly with this transformed therapeutic landscape through two principal findings.

The results of the first two studies collectively indicate that the refined Aβ–, GAMLSS-based, comprehensive neuropsychological norms improve early detection and characterization of cognitive impairment –particularly that related to AD–, thereby better informing the use and interpretation of

biomarkers, and thus improving the selection of appropriate candidates and the assessment of clinical efficacy of therapeutic interventions.

The third work identified a novel FTLD-causing variant in the *CHMP2B* gene through comprehensive WES in a family with a history of diverse neuropsychiatric disorders of unknown etiology. This case study provides illustrative insights into the diagnostic potential of genetic testing in FTLD, with far-reaching implications for the patient, their relatives, and the research field, and informs the ongoing debate on the best-practice standards for genetic testing in non-ALS FTLD-S.

7.1. The transformed therapeutic landscape of AD

Building on aducanumab's 2021 precedent, results from the phase III trials of two anti-Aβ monoclonal antibodies – lecanemab (CLARITY AD) and donanemab (TRAILBLAZER-ALZ 2)– were published in 2022 and 2023, respectively. Both were multicenter, randomized, double-blind, placebo-controlled 18-month phase III trials comparing intravenous anti-Aβ therapy (lecanemab every two weeks; donanemab every four weeks) versus placebo in participants with early symptomatic (MCI or mild dementia), biomarker-confirmed AD.(202,203)

Both lecanemab and donanemab phase III trials met their primary clinical endpoint, which was the 18-month change from baseline in the composite cognitive-functional measures Clinical Dementia Rating-Sum of Boxes (CDR-SB;(208) range 0-18, with higher scores reflecting greater cognitive-functional impairment) and Integrated Alzheimer's Disease Rating Scale (iADRS;(209) range 0-144, with lower scores reflecting greater cognitive-functional impairment), respectively. In particular, compared to placebo, the lecanemab-treated group showed a 27% slower decline in the CDR-SB, and the donanemab-treated low-to-medium tau PET subgroup presented a 35% slower reduction in the iADRS. Both drugs also met all their secondary cognitive and functional outcomes, demonstrating significant slowing of symptomatic decline. As for biological efficacy, both drugs substantially lowered Aβ levels as measured on amyloid PET (71% vs 86% amyloid PET signal reduction for lecanemab and donanemab, respectively).(111,202,203,210)

The positive association demonstrated in the lecanemab and donanemab phase III trials between Aβ clearance and clinical efficacy, as well as the observed reductions in further secondary biological endpoints involving biomarkers of tau and neurodegeneration, substantiate the long-standing amyloid cascade hypothesis.(111,122,202,203) This, in turn, may support the validity of our approach to normative sample selection, as implemented in the first study of this thesis, in which we excluded individuals with biomarker evidence of brain Aβ pathology without considering other AD biomarkers (i.e., markers of tau or neurodegeneration).

Both lecanemab and donanemab were associated with adverse events. Infusion-related reactions occurred in both lecanemab- and donanemab-treated patients. Nevertheless, the most concerning adverse event of these –and all anti-Aβ monoclonal antibodies to date– are amyloid-related imaging abnormalities (ARIA), consisting of either edema (ARIA-E) or hemorrhagic imaging findings (ARIA-H, including microhemorrhages, superficial siderosis, and rarely, macrohemorrhages). The risk of ARIA is higher in individuals with previous cerebral hemorrhage, advanced small vessel disease, hypertension, or on anticoagulant medication. A higher risk of ARIA has also been observed in *APOE ε4* carriers, highest in *ε4/ε4* homozygotes due to the higher frequency of amyloid angiopathy in these individuals.(111,202,203,210)

Appropriate use recommendations have been issued for treatment with both lecanemab and donanemab, according to which patient eligibility relies on a diagnosis of MCI or mild dementia due to biomarker-confirmed AD. Specifically, recommendations for the appropriate use of lecanemab and donanemab state that treatment candidates should have brain Aβ pathology confirmed by either amyloid PET or CSF biomarkers. Nevertheless, they also acknowledge the great potential of blood-based biomarkers to enhance access to treatment in the near future.(211,212)

On the other hand, lecanemab and donanemab appropriate use recommendations advise that treatment not be given to individuals with an elevated risk of ARIA due to anticoagulant medication or as established by a baseline MRI within one year of treatment initiation (e.g., 1 macrohemorrhage,

>4 microhemorrhages, superficial siderosis, 1 major territory infarction, >2 lacunar infarcts, advanced white matter disease, vasogenic edema). Most ARIA are clinically silent, and therefore serial MRIs are required for safety monitoring during treatment with lecanemab and donanemab. The guidelines also recommend *APOE* genotyping before treatment initiation to inform the risk of ARIA.(211,212)

By early 2026, either lecanemab or donanemab (or both) have received regulatory approval in approximately 50 countries worldwide. In Europe, the EMA recommended marketing authorization for lecanemab and donanemab, which were granted in April 2025 and September 2025, respectively, following a re-examination of the Committee for Medicinal Products for Human Use (CHMP)'s initial negative opinions on both therapies due to safety concerns. (213–215)

At present, phase III trials are ongoing for many other potentially disease-modifying therapies for AD, including several additional anti-Aβ antibodies. Just as CLARITY AD and TRAILBLAZER-ALZ 2, many of the ongoing phase III trials are tailored to individuals in the early symptomatic stages of AD (i.e., MCI and mild dementia).(111,204)

Nevertheless, AD-targeted therapies are expected to be more efficacious when initiated in the earliest stages of the disease, before widespread pathology, and thus individuals with preclinical AD (either with or without subjective cognitive decline) are now the target population for some promising preventive phase III trials (e.g., lecanemab [AHEAD 3-45](216) and donanemab [TRAILBLAZER-ALZ 3](217)). However, the efficacy of these drugs to prevent or delay symptom onset is still being investigated, and no preventive AD therapies have been approved to date.(111,204)

7.1.1. Appropriate candidate selection for AD-targeted therapies

As noted in the first two sections of this thesis, AD is characterized by a long preclinical stage spanning up to 20 years,(42–44) with the prevalence of clinically silent Aβ pathology increasing with age.(156) Accordingly, biomarker

screening for AD pathology in cognitively unimpaired older adults would test positive in a substantial number of individuals with preclinical AD, who would not qualify for treatment with lecanemab nor donanemab (i.e., tailored to early symptomatic AD stages).(111,218,219)

In the absence of demonstrated clinical efficacy of preventive AD treatments or of beneficial outcomes from biomarker disclosure in preclinical AD populations, biomarker testing is not recommended for cognitively unimpaired individuals in the clinical setting.(24,25,218) This underscores the need for a comprehensive neuropsychological assessment sensitive to the earliest stages of cognitive impairment, thereby identifying appropriate candidates for biomarker testing and, potentially, for anti-Aβ therapy.(28,138,219)

The first two studies of this thesis directly respond to the need to detect the earliest objectively measurable cognitive changes. In particular, the results of the second study indicate that one in five individuals currently classified as CN might be better diagnosed with MCI, and thus gain earlier access to biomarkers and, eventually, to treatment.

However, even with documented cognitive impairment, AD biomarker interpretation is not straightforward. For the same reason that cognitively unimpaired older individuals may obtain positive AD biomarker results, AD biomarkers may also be positive in individuals with cognitive impairment primarily driven by non-AD causes, including both copathologies and comorbidities.(28,126,218,219)

Definitive biomarkers are lacking for most non-AD neuropathologies (e.g., LBD, FTLD, LATE-NC, AGD, ARTAG), and, in any case, biomarker staging does not correlate directly with their degree of contribution to the patient's clinical symptoms. Likewise, the existence of comorbidities (e.g., medications, depression, obstructive sleep apnea) complicates the attribution of clinical symptoms. This warrants a thorough diagnostic evaluation of cognitive impairment, including a detailed medical history, neurological examination, routine laboratory workup, and structural brain imaging.(28,126,218,219) In this regard, a comprehensive neuropsychological assessment is crucial to

characterize the profile of cognitive impairments, enabling interpretation of AD biomarkers and further informing the differential diagnosis of the likely underlying cause(s) of the patient's symptoms.(37,126)

The first study of this thesis provides normative data for a comprehensive battery of neuropsychological measures. As anticipated based on the approach of selecting an Aβ− normative sample, the results of the second study suggest that our norms are notably sensitive to impairments in episodic memory, which is widely established as the hallmark cognitive deficit in AD.(51,52,220) In particular, memory impairments predominantly accounted for CN-to-MCI diagnostic reclassifications under our norms, compared with current standards. The validity of such reclassifications was supported by the high proportion of Aβ+ individuals in the CN-to-MCI group. Furthermore, a faster rate of decline was observed among Aβ+ individuals in the CN-to-MCI group compared to Aβ+ individuals classified as CN by both norms, although this difference did not reach statistical significance, likely due to both the reduced subsample size and the similarly progressive trajectory among Aβ+ CN individuals (i.e., preclinical AD). Thus, predictably, findings suggest that our Aβ− norms are particularly sensitive to early cognitive changes associated with AD.

In contrast, the lack of biomarker or autopsy confirmation for non-AD neuropathologies represents a limitation for both the development of neuropsychological reference values (which may be biased by the unintended inclusion of individuals with preclinical non-AD neuropathologies in the normative sample) and the evaluation of our norms' utility for detecting non-AD causes of cognitive impairment. With respect to the latter limitation, the observation that Aβ− individuals in the CN-to-MCI group showed steeper longitudinal cognitive decline than Aβ− individuals classified as CN by both norms provides preliminary evidence that our norms may also offer added value in detecting non-AD causes of cognitive impairment. However, this difference in the Aβ− longitudinal subset did not reach statistical significance either, possibly due to a combination of reduced statistical power and the expected mixture of non-AD etiologies in the Aβ− CN-to-MCI reclassified group, encompassing both progressive neurodegenerative pathologies and

non-progressive comorbidities. Therefore, there is not sufficient evidence to draw conclusions about our norms' performance in detecting cognitive impairment of non-AD etiology.

As demonstrated by the situations outlined above, the requirement for AD biomarker confirmation for treatment eligibility should not be interpreted as implying that biomarkers suffice for candidate selection, since individuals at the preclinical stage of AD, or with coexisting non-AD causes primarily driving cognitive impairment, are currently ineligible for approved anti-Aβ therapies.(111) A comprehensive clinical evaluation, including an accurate neuropsychological assessment, is thus crucial for the indication and interpretation of AD biomarkers, and therefore for the selection of candidates for approved anti-Aβ therapies.

Notably, individuals at moderate-to-severe dementia stages of AD are likewise not candidates for approved anti-Aβ antibodies. Building on this, individuals with atypical AD phenotypes stand to benefit from comprehensive neuropsychological assessment to identify eligible candidates for AD-targeted therapies. For instance, patients with early-stage lvPPA might rather appear to be on the moderate-to-severe dementia range due to their prominent language deficits when relying on measures of global cognitive functioning (e.g., MMSE), thereby erroneously precluding them from treatment consideration.(28)

The normative data derived in the first study provide a thorough characterization of neuropsychological performance beyond memory and across the main cognitive domains. This, combined with the biomarker-sensitive norming approach and the improved precision and flexibility offered by the GAMLSS framework, renders our norms potentially useful in the early detection of non-amnestic presentations of AD. In fact, although the analyses in the second study were not specifically designed to assess the norms' diagnostic performance for each AD phenotype, the results suggest that the clinical value of the norms may extend to other cognitive domains beyond memory. In particular, a stepwise pattern of neuropsychological performance, similar to that in memory tests, was also observed in language and some attention/executive measures, contributing secondarily to CN-to-MCI reclassifications.

Concerning visuospatial skills, caution is warranted when interpreting the lack of the expected stepwise performance across the four diagnostic groups in the second study: because measures of visuospatial function were not taken into account in assigning actuarial diagnoses due to a high proportion of missing data, the absence of such a pattern is expected and reflects the inherently visuospatial-agnostic nature of the analyses. Thus, collectively, these findings support the potential value of our norms for the early diagnosis of individuals with atypical, non-amnestic presentations of AD; however, these interpretations should be viewed as preliminary given the non-targeted analyses.

Clinical trials tailored to early symptomatic and preclinical AD stages may likewise benefit from our norms' enhanced detection of early cognitive changes associated with Aβ pathology, potentially reducing sample size requirements while maximizing the detection of therapeutic effects.

In summary, our results indicate that the Aβ−, GAMLSS-based neuropsychological norms derived in this thesis may streamline diagnostic and therapeutic decisions and inform clinical trial design across the phenotypic spectrum of AD and throughout clinical stages of disease progression.

The findings of the first two studies may also have broader implications. By enabling more timely and accurate diagnosis, refined neuropsychological norms can improve patient care irrespective of whether AD is ultimately regarded as the main cause of their cognitive impairment, or whether they qualify for either disease-modifying therapies or clinical trials. For example, an accurate characterization of the profile of cognitive deficits can guide non-pharmacological interventions targeted to the patient's specific impairments (e.g., language therapy and communication-enhancing strategies in patients with lvPPA or svPPA due to AD or FTLD, respectively).(37)

7.1.2. Clinical efficacy assessment in AD-targeted therapies

Both the FDA and the EMA have issued clinical indications for initiating treatment with lecanemab and donanemab in patients with MCI or mild dementia due to AD. By contrast, the FDA has not specified when to stop

treatment aside from adverse events or donanemab-induced amyloid clearance,(221,222) whereas the EMA explicitly recommends treatment discontinuation for lecanemab (or clinical consideration to discontinuing treatment with donanemab) if the patient progresses to moderate AD dementia on 6-monthly cognitive testing and symptom evaluation.(213,214)

In this context, serial longitudinal neuropsychological assessments, ideally employing normed scores sensitive to cognitive decline associated with AD, could provide a rigorous basis for tracking clinical disease progression and thus for deciding whether to discontinue treatment with lecanemab or donanemab.(111,138) The findings of the first two works indicate that the neuropsychological norms derived in this thesis are sensitive to the early cognitive changes in AD, and could therefore be particularly useful to inform clinical decisions on treatment discontinuation in our country.

In the field of clinical trials, the recent approval of the first disease-modifying therapies for AD has reignited the debate on the need for AD trials to include measures of clinically meaningful change. Perceptions of what constitutes a clinically meaningful change vary across different AD stakeholders (i.e., patients, care partners, healthcare providers, researchers, sponsors, and regulatory agencies) and across different AD stages. Within the ongoing discussion, there is growing recognition of the importance of AD trials incorporating standardized sets of outcomes relevant to patients' and caregivers' treatment-related needs and priorities across the AD continuum, thereby enhancing the relevance, responsiveness, and comparability of clinical trials.(223–226)

In the What Matters Most study, semi-structured interviews with individuals from preclinical to advanced AD stages and their care partners elicited current symptom burden, as well as their most desired treatment outcomes. All participants (including those with preclinical AD) concurred that memory deficits were the most concerning AD symptoms at the time, followed by communication and language difficulties. As for the identified treatment-related needs, preserving cognitive abilities –particularly slowing or preventing memory loss– was the most important outcome for two-thirds of

the participants, above improving daily functioning, curing AD or removing plaques and tangles.(227)

As scientific knowledge of AD has advanced, clinical trial efforts have progressively been directed toward earlier, preclinical-to-early-MCI stages of AD. Updated FDA (2024) recommendations on the outcome measures considered for drug efficacy in early AD are available,(228) while a revision of the 2018 EMA guidelines is expected in the fourth quarter of 2026.(229,230)

The FDA guidelines acknowledge that, while cognitive and functional measures remain valid primary outcomes for trials in the MCI-to-dementia stages of AD (either as co-primary endpoints or through composite scales), they may not be so in the earliest AD stages, which, by definition, have no or minimal cognitive-functional impairments. Specifically, the FDA argues that many of the functional measures used in the later disease stages would lack sensitivity to the subtle, yet clinically meaningful changes of early AD, thereby requiring longer trial durations to measure progression.(228)

Instead, in patients in the preclinical-to-early-MCI stages of AD, the FDA states that they would consider evidence of clinically meaningful change a convincing beneficial effect on cognition alone, as assessed by a sensitive neuropsychological test or, ideally, a battery of sensitive neuropsychological tests across multiple cognitive domains. They also state that such an effect on neuropsychological measures would be expected to be supported by a similarly convincing effect on biomarker outcomes.(228) At this point, it is worth reiterating that, as indicated by the results of the second study, the neuropsychological norms derived in this thesis are sensitive to cognitive changes associated with the earliest stages of AD, particularly –but not only– deficits in episodic memory.

Neuropsychological evaluation enables comprehensive assessment of an individual's performance across the main cognitive domains and relative to a demographically adjusted normative sample. However, differences in language and cultural background hinder the interpretability and comparability of neuropsychological assessments. To address this, harmonization of

neuropsychological test batteries has been proposed to improve the accuracy and consistency of cognitive assessments across cultures and languages.(37,226) In this respect, the set of cognitive tests for which normed scores were derived in the first work of this thesis aligns with efforts to harmonize neuropsychological assessment in Europe.(157)

Taken together, these data indicate that the neuropsychological norms derived in this thesis could contribute to the comprehensive and standardized tracking of cognitive abilities under approved or trial AD therapies, while adhering to patient-defined priorities that place memory as the most valuable treatment outcome.(227) In addition, our neuropsychological norms could also contribute to the assessment of clinical efficacy of non-pharmacological interventions (e.g., domain-specific cognitive training).

7.2. The reshaped landscape of FTLD gene-targeted therapies

Following successful phase I-II findings of tofersen,(124) an ASO that decreases synthesis of the toxic SOD1 protein in *SOD1*-ALS, the results from the phase III trial (VALOR) were published in 2022. This was a multicenter, randomized, double-blind, placebo-controlled phase III trial in adults with *SOD1*-ALS comparing intrathecal bolus administration of tofersen (eight doses over a 24-week treatment period, followed by a 4-to-8-week monitoring period, then followed by an open-label extension phase) versus placebo.(205)

The primary clinical endpoint of the phase III trial of tofersen was the change from baseline at 28 weeks in the total score on the ALS Functional Rating Scale–Revised (ALSFRS-R; range, 0 to 48, with higher scores reflecting greater functional ability) among participants with predicted faster disease progression. Secondary biological endpoints included change from baseline in the CSF concentration of the SOD1 protein and in the plasma concentration of NfL; additional secondary clinical endpoints were also assessed. Tofersen met both secondary biological endpoints of lowering CSF concentrations of SOD1 and plasma levels of NfL at week 28, but it met neither primary nor secondary clinical endpoints and was not free of adverse events.(205)

Based on the large, robust reduction in the plasma NfL concentrations over the 28-week phase III trial period, tofersen received FDA's accelerated approval for *SOD1*-ALS in April 2023.(231)

While tofersen's phase III trial failed to meet its primary and secondary clinical endpoints, the later analysis of its open-label extension provided support that tofersen slows disease progression in *SOD1*-ALS.(232) Based on combined results from the double-blind phase and the open-label extension, in February 2024, the EMA issued a positive opinion recommending tofersen's marketing authorization under exceptional circumstances, acknowledging the rarity of the *SOD1* pathogenic gene variant, which is only found in 2% of all ALS cases. Marketing authorization was subsequently granted by the European Commission in May 2024.(233)

Besides tofersen, multiple gene therapies are currently under investigation for both ALS and non-ALS FTLD.(234,235)

7.2.1. Moving toward best-practice standards for diagnostic genetic testing in FTLD

Although access to genetic testing has been endorsed since 2021 as a fundamental right for all individuals diagnosed with ALS,(236) prevailing clinical practice guidelines until recently either did not address genetic testing for ALS at all,(237) or recommended that it be primarily restricted to familial ALS cases.(238) This had resulted in genetic testing not being consistently offered to people living with ALS.(239–241)

The emergence of genotype-targeted therapies for ALS has catalyzed the development of specific clinical practice guidelines on diagnostic genetic testing for individuals with ALS, which were published in September 2023. In short, departing from previous practices, the 2023 evidence-based consensus guidelines advocate for offering genetic testing to all individuals with ALS, and recommend a single-step testing approach that includes at least the *C9orf72* repeat expansion, *SOD1*, *FUS*, and *TARDBP*, as opposed to sequential gene testing.(206)

Diagnostic genetic testing is an essential prerequisite for eligibility for gene-targeted therapies and clinical trials.(198) One of the rationales underlying the 2023 guidelines recommendation of universal offering of genetic testing to individuals with ALS is that pathogenic gene variants may be found not only in familial ALS, but also in apparently sporadic cases. Yet another explicitly mentioned reason is that patients with ALS perceive access to genetic testing as beneficial, regardless of familial antecedents or test results.(206) These same rationales could arguably apply to non-ALS FTLD.

As detailed in Section 2, pathogenic gene variants may be found in 50-70% of familial FTLD cases, and in up to 20% of apparently sporadic FTLD (**Figure 4**).(74,145,192–195) Moreover, individuals living with non-ALS FTLD may have a myriad of other legitimate reasons for pursuing genetic testing, including the establishment of a definitive diagnosis and the understanding of the underlying disease causes, as well as the ascertainment of the possibility that it can be passed to their loved ones.(242)

At the time of submission of this thesis, clinical guidelines on genetic testing for non-ALS FTLD-S are lacking, and practices regarding the offering of genetic testing vary across clinicians and settings.(92,198,207) However, expert recommendations increasingly support offering genetic testing to all individuals with any FTLD-S, irrespective of their apparent family history.(92,192,198) In this regard, in October 2025, the Association for Frontotemporal Degeneration (AFTD) issued a new recommendation to expand access to genetic testing to all people with FTLD-S upon diagnosis.(243)

As for the recommendation for single-step multi-gene testing in individuals with ALS, the 2023 clinical practice guidelines note that this diagnostic approach is more cost- and time-efficient than sequential gene testing.(206) In this line of reasoning, it is increasingly recommended that a comprehensive genetic testing strategy be adopted for individuals with non-ALS FTLD-S as well.(198,244) The recent AFTD recommendation also supports comprehensive genetic testing –including but not limited to *C9orf72*, *GRN*, and *MAPT*–, indicating a range of implications for the patient that extend from reaching a definitive diagnosis to aiming for gene-targeted therapies. In this respect,

the AFTD advocates for a thorough genetic testing that goes beyond genes with readily available genotype-targeted therapies or clinical trials, arguing that knowing an individual's genetic status in advance may foster access to gene-targeted treatment should it eventually become available. The AFTD also notes several implications for at-risk family members and the research field.(243)

Comprehensive genetic testing may be performed with a targeted NGS panel of genes of interest; alternatively, WES and WGS can be used. Notably, WES and WGS enable future data reanalysis in light of new information about the proband or novel disease-gene association discoveries, while they also provide an opportunity to discover new disease-causing genes.(146,198)

With respect to the third work included in this doctoral thesis, the approach adopted on further study of the index case, given the diagnostic uncertainty during follow-up, aligns with these growing recommendations that the offering of diagnostic genetic testing for individuals with FTLD-S should be both universal and comprehensive.

To recap briefly, the index case, who presented with a slowly progressive bvFTD syndrome, proved negative upon initial *C9orf72* testing. The isolated testing for the *C9orf72* repeat expansion in the proband is consistent with previous practice recommendations that *C9orf72* testing should be offered to all individuals of European ancestry with any FTLD-S, whereas a broader genetic study should be primarily reserved for familial cases.(189,245) As for the family history, whether it should be regarded as positive or negative may be subject to debate depending on whether first- only or second-degree family members are included, as well as on whether any neuropsychiatric history is considered. As a reminder, the proband's father had died from late-onset dementia of uncertain etiology. Beyond the first-degree family, there were several antecedents of neuropsychiatric disorders of unknown etiology, including two suicide cases. Of note, the index case manifested symptoms 4 years earlier than his twin; therefore, the latter did not initially contribute to the family history.

Even if the family history had been deemed positive from the outset, a sequential genetic testing strategy until the pathogenic *CHMP2B* variant was identified would have been prohibitively time-consuming, and therefore not feasible in routine clinical practice.

Irrespective of the strategy adopted, diagnostic genetic testing should always include a discussion of all possible outcomes and their potential familial implications.(198) Regarding implications for the family, not only do they have to cope with the diagnosis of a devastating disease in their close relative, but knowledge about its genetic component may occur shortly afterward. Even though this information entails profound emotional stress, it also has potential benefits to the family. For symptomatic relatives, genetic information can help clarify their diagnosis, as occurred in the third study of this thesis, where the proband's affected sibling had been misdiagnosed with DLB before genetic testing. Asymptomatic at-risk family members may benefit from adequate genetic counseling and testing to determine their own risk and make informed decisions, including life planning and reproductive choices.(246)

A genetic diagnosis also has multiple research implications. First, it provides clinical trials with appropriate FTLD-confirmed candidates, thereby enhancing the detection of beneficial therapeutic effects.(76,243) Second, examining the consequences of gene malfunction on biological processes contributes to understanding the underlying mechanisms of disease and identifying further potential targets for therapeutic development.(243,247) Finally, both individuals with genetically confirmed FTLD and their at-risk family members may participate in observational studies that aid in the characterization of the disease's natural history. For over a decade, various international multicenter efforts (e.g., the Genetic Frontotemporal dementia Initiative [GENFI] –of which our center is a participating site–, the combined Advancing Research and Treatment in Frontotemporal Lobar Degeneration [ARTFL] – Longitudinal Evaluation of Familial Frontotemporal Dementia Subjects [LEFFTDS] Longitudinal Frontotemporal Lobar Degeneration [ALLFTD] research consortium) have recruited large cohorts of symptomatic and presymptomatic participants with genetic forms of FTLD, with the objective of

better understanding the earliest clinical, biofluid and neuroimaging changes, and the progression of the disease.(76,147)

Our case report illustrates the potential of low-threshold comprehensive genetic testing to improve the yield and timeliness of FTLD diagnosis, with multiple implications for the patient, their family, and the research field. The third study included in this thesis adds to the growing recommendation that comprehensive genetic testing be offered to all individuals with some form of FTLD-S.

Beyond the limitations outlined in the third work, which pertain specifically to the case study, there are common limitations associated with widespread, comprehensive genetic testing that merit comment.

A first limitation is that individuals living with an FTLD-S may have cognitive and/or behavioral impairments that could affect their capacity to make genetic testing decisions. In such cases, decision-making is often delegated to their care partner. However, it should be borne in mind that the care partner is frequently a biological relative, and therefore their decision may be influenced by concern about their own risk. The clinician must take care that the decision is aligned with the best interests of the patient and, whenever possible, the patient's autonomy must be respected.(198)

Another issue is that testing a greater number of genes is associated with an increased likelihood of indeterminate results.(198,245,248) According to current guidelines, gene variants are classified as pathogenic, likely pathogenic, benign, likely benign, or variant of uncertain significance (VUS) based on variant frequency in population and disease-specific databases, along with clinical information, computational predictions of the variant's molecular effects, and functional studies.(249) Uncertain genetic test results (i.e., VUS) pose challenges for diagnosis and clinical management of patients, and for risk assessment for biological relatives.(248) The VUS rate in ALS-targeted gene panels has been reported to be up to 12-30%.(250) Should a VUS be identified, in silico predictions, functional analyses, and familial segregation of the variant with disease may inform timely reclassification;

otherwise, periodic reevaluation may enable future VUS resolution in the light of new evidence.(198,248,251) While widespread genetic profiling of clinical populations increases the likelihood of VUS outcomes, it may, in turn, reduce uncertainty surrounding variant classification by contributing to disease-specific databases.(249,252)

7.3. Genetic counseling in neurodegenerative diseases presenting with cognitive and/or behavioral impairment

> *"Genetic counseling is the process of helping people understand and adapt to the medical, psychological, and familial implications of genetic contributions to disease."*
>
> National Society of Genetic Counselors' Task Force Report, 2006, p.77(253)

The offering of diagnostic genetic testing to individuals with FTLD and other neurodegenerative diseases should always be accompanied by appropriate genetic counseling.(254) Two key components can be distinguished within the genetic counseling process. The first one involves handling genetic information within the family, including the provision of risk assessment for relatives. As noted above, the implications of a genetic diagnosis extend far beyond the patient and concern their family members; thus, it is strongly advised that at-risk relatives be involved in decisions regarding diagnostic testing. The second aspect of genetic counseling is the genetic testing process itself.(192,198,242,255)

Should a pathogenic (or likely pathogenic) gene variant be found in a symptomatic individual, their at-risk family members may benefit from predictive genetic counseling to understand their own risk and make informed decisions about whether to be tested themselves.(246,256) Of note, in cases where genetic testing for an affected individual yields a VUS outcome, testing of asymptomatic relatives is recommended against except for VUS resolution (i.e., familial segregation analysis).(198)

Predictive genetic counseling for hereditary neurodegenerative diseases began in 1994 with the guidelines for Huntington's disease.(257) To date, the 2013

updated recommendations for Huntington's disease remain the gold standard for predictive genetic counseling and testing for neurodegenerative diseases.(201,258) Predictive genetic counseling for neurodegenerative diseases, as specified by Huntington's disease protocol, is a process that develops over multiple sessions and involves a multidisciplinary team, which may include a neurologist, a clinical geneticist, a genetic counselor, and a psychiatrist or psychologist. As for the at-risk individual, it is advised that they be accompanied by a support person throughout the genetic counseling process.(258)

As with diagnostic counseling, predictive counseling should cover all possible scenarios arising from genetic testing, including consequences for further family members. Making an informed testing decision requires a clear understanding of the inheritance pattern, the penetrance, the expected age of onset, and the possible clinical presentations of the disease (which may be different from that of their affected relative), given that one is a carrier of the pathogenic gene variant. An informed decision also depends on knowledge about the availability of reproductive options, approved therapies, clinical trials, and the prospects for care. Common reasons to opt for testing include uncertainty management, life planning, implications for family members, and reproductive choices. However, the genetic counseling process also involves addressing the right of those at-risk family members who don't want to know their genetic status. In this regard, extreme caution is advised when one's right to know comes into conflict with others' right not to know (i.e., when an individual's positive test result would imply that another individual is necessarily an obligate carrier of the genetic condition).(201,258)

Regardless of whether the at-risk individual decides to undergo testing or not, offering them to enroll in observational studies enables them to receive clinical follow-up while finding purpose in their risk status by contributing to research. For individuals who opt for genetic testing, tailored post-test counseling and psychological support must be made available to facilitate adjustment, regardless of test results.(201,256,258)

Reproductive options for couples where one partner is at risk of a genetic condition include pursuing pregnancy without genetic testing, prenatal

genetic diagnosis (followed by the couple's decision on whether to opt for abortion), preimplantation genetic diagnosis, donor insemination, and adoption. Importantly, both prenatal and preimplantation diagnosis are possible without the at-risk parent having to know their own genetic status (i.e., exclusion prenatal and preimplantational genetic diagnosis).(258)

The 2023 evidence-based consensus guidelines for ALS provide standardized pre-test and post-test genetic counseling recommendations for all individuals with ALS.(206) In the meantime, evidence-based clinical guidelines on genetic counseling for individuals with non-ALS FTLD and their at-risk relatives remain elusive.(242,255)

Although this thesis focuses on the diagnostic utility of genetic testing in FTLD, with the derived need for genetic counseling, genetic counseling is becoming increasingly relevant in other ADRD as well, particularly with the emergence of anti-Aβ monoclonal antibodies for AD.

Both lecanemab and donanemab appropriate use recommendations advocate for *APOE* genotyping of all patient candidates before treatment initiation, given the strong association between the number of *APOE ε4* alleles and the treatment-related risk of ARIA.(211,212) Of particular note here is the fact that being an *APOE* ε4/ε4 homozygote constitutes an exclusion criterion for lecanemab and donanemab in Europe, the UK, and Australia; by contrast, the US FDA authorizes treatment with lecanemab and donanemab irrespective of *APOE ε4* carrier status.(111) *APOE* genotyping results thus inform effective communication with patients and their caregivers about the risks associated with anti-Aβ therapies, and may render a patient ineligible or require intensified safety monitoring during treatment (e.g., additional MRIs).(111,259)

Beyond the challenges of effectively conveying to cognitively impaired individuals the probabilistic risk of treatment side effects based on their *APOE* genotype, ethical and practical challenges also arise from the dissemination of genetic information within the family.

As explained in the introduction, a strong dose-dependent association also exists between the *APOE ε4* allele status and the lifetime risk of AD, with

increased risk observed for individuals carrying one *ε4* allele, and the highest risk for *APOE ε4/ε4* homozygotes.(48) Notwithstanding, current clinical practice guidelines recommend against *APOE* testing for the assessment of future AD risk, since being an *APOE ε4* carrier has long been considered neither necessary nor sufficient to develop the disease.(260)

APOE-derived information on patient eligibility for anti-Aβ therapies and the likelihood of ARIA is accompanied by the sharing of genetic information within the family. The most extreme instance is when a patient proves to be an *APOE ε4/ε4* homozygote (rendering them potentially ineligible for treatment) and, therefore, all of their children become obligate carriers of at least one *APOE ε4* allele.(261) In biomarker-confirmed AD patients, the discussion prior to *APOE* genotyping must include the potential implications for the patient and their family members (medical, psychological, social), and post-test counseling must be made available in all cases.(259,261,262) However, the appropriate use recommendations for lecanemab and donanemab provide no specific guidance on pre-test or post-test counseling regarding the implications of *APOE* testing.(211,212)

Millions of individuals diagnosed with MCI or mild dementia due to AD are potential candidates for Aβ—lowering therapies, and would thus theoretically qualify for *APOE* genetic counseling and testing.(261) As noted in the introduction, approximately 40-65% of AD patients are *APOE ε4* carriers.(50) Offering *APOE* testing to all potential patient candidates would therefore have implications for numerous biological family members and thus further overload an already critical shortage of trained genetic counselors.(263)

The new indication for *APOE* genotyping to inform Aβ-lowering treatment options necessitates updating guidelines on genetic counseling and testing for AD. Remarkably, among the many individuals in the early symptomatic stages of biomarker-confirmed AD, prior studies have estimated the eligibility rate for anti-Aβ therapies to be as low as 5-8% upon application of the clinical trial inclusion (e.g., MMSE score, CDR global score) and exclusion criteria (e.g., MRI findings, chronic comorbid conditions, and past medical history), without considering *APOE* genotype.(264) In this context, to maximize the benefits

while minimizing the ethical and practical drawbacks of *APOE* testing, I would suggest considering *APOE* genotyping be restricted to those patient candidates interested in receiving anti-Aβ drugs and without other a priori exclusion criteria. Although some might argue that this stepwise approach would objectionably increase the waiting time for treatment, from my perspective, it would avoid unnecessary psychological impact of *APOE* testing results on family members, while also mitigating the overburden of healthcare systems.

8. CONCLUSIONS

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1) Comprehensive neuropsychological norms derived jointly from selecting Aβ− CN adults and applying the GAMLSS framework, and accounting for age, education, and sex, enable earlier detection of cognitive impairment related to AD. These norms offer readily implementable standards for neuropsychological assessment in our country, which may inform clinical decision-making and trial design.

2) Comprehensive genetic testing upon diagnosis or suspicion of some FTLD-S holds great potential to improve the yield and timeliness of FTLD diagnosis, with far-reaching implications for the patient and their family, as well as for the research field. Our findings build on the growing literature supporting that all individuals with suspected FTLD should be offered comprehensive genetic testing.

9. FUTURE DIRECTIONS

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Future clinical and research directions emerge naturally from both the thesis's limitations and the relentless evolution of the field.

The thesis findings suggesting that our norms enhance the detection of AD-related cognitive impairment should be replicated in an independent cohort sample to provide more robust evidence of diagnostic performance. In particular, future studies using longitudinal analyses and base-rate approaches should intend to obtain a more precise estimate of specificity. Future research should also pursue targeted exploration of our norms' diagnostic capabilities across distinct AD phenotypes, as well as their diagnostic value from a sex perspective.

As discussed earlier, the neuropsychological norms derived in this thesis do not capture the linguistic and cultural heterogeneity of our country, nor do they include assessments of social cognition. To improve the representativeness and applicability to broader populations, similar norms should be defined that consider the language and cultural diversity both within and across countries. Future studies should also incorporate cross-culturally validated measures of social cognition to enhance neuropsychological diagnostic capabilities. (157,265)

As biomarkers for LBD and FTLD become increasingly available, future works could examine our norms' performance in detecting cognitive impairment driven by non-AD neuropathologies. Likewise, future research efforts could apply the GAMLSS framework to derive neuropsychological norms from normative samples without biomarker evidence of AD, nor of non-AD neuropathologies (i.e., LBD, FTLD).

As the field shifts toward earlier detection of cognitive decline in preclinical AD, with emerging operational criteria for objective subtle cognitive decline (i.e., stage 2),(266) future studies could use the sensitive, AD-negative norms developed in this thesis to validate candidate classification approaches.

Along the same lines of early detection efforts, the norming method used in this thesis, of combining biomarker-negative normative samples and the GAMLSS framework, could also be used to derive reference values for emerging digital biomarkers, which promise to provide sensitive, ecological assessments of cognitive functioning.

Another important, albeit distinct, direction pertains to the use of genetic tools. Building on the growing recommendation that comprehensive genetic testing be offered to all individuals with an FTLD-S, it is a key priority to develop evidence-based clinical practice guidelines for diagnostic genetic testing in individuals with non-ALS FTLD. Such future standards should incorporate recommendations for pre-test and post-test genetic counseling, as well as good clinical practice for predictive counseling and testing for at-risk family members.

In closing, healthcare systems need to equip themselves with adequately trained professionals to overcome the new challenges they face.

With the advent of anti-Aβ therapies, healthcare systems are urgently compelled to establish the necessary infrastructure to enable biomarker detection, treatment administration, and safety monitoring. However, specialized staff is also needed to meet the foreseeable increase in healthcare-seeking for cognitive concerns.(122,111,267) Trained neuropsychologists and neurologists are best placed to provide comprehensive clinical evaluations that enable early and accurate detection of AD and other neurodegenerative diseases presenting with cognitive and/or behavioral impairment.

In parallel, as the demand for genetic counseling in ADRD increases rapidly, so does the need to provide non-genetics professionals – including neurologists – with additional training to overcome the limited availability of genetic counselors. Irrespective of the above, to enable well-informed decision-making regarding genetic testing, sufficient visit time must be allowed so that both cognitively impaired patients and their unimpaired family members can understand complex genetic concepts and the possible test outcomes. Ultimately, the incorporation of genetic counseling and testing into clinical

practice will add further tension to a comparable shortage of clinicians with expertise in neurodegenerative diseases presenting with cognitive and/or behavioral impairment.(198,261,262)

10. BIBLIOGRAPHY

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11. ANNEXES

11. ANNEXES

11.1. Supplementary material for article 1: Development of amyloid-negative neuropsychological norms using GAMLSS

Supplementary Methods

Cerebrospinal fluid (CSF) biomarker analyses by center

Hospital de la Santa Creu i Sant Pau

CSF was collected and processed in polypropylene tubes following international recommendations.[51,52] The first 2 mL of CSF were transferred to the general laboratory for cell count, and glucose and protein level analyses. Another 15-20 mL volume was transferred to our laboratory, where samples were processed and aliquoted within the first 2 hours after the lumbar puncture. Aliquots were stored at −80ºC until analysis. Core Alzheimer's disease (AD) biomarkers [amyloid β 1-42 (Aβ42), amyloid β 1-40 (Aβ40), total tau (t-tau), and phosphorylated tau at threonine-181 (p-tau)] were measured in all participants. Biomarker cutoffs were derived from 96 participants of the Sant Pau Initiative on Neurodegeneration (SPIN cohort)[53] with available 18F-Florbetapir positron emission tomography (PET) using the Lumipulse G System. Participants had mild cognitive impairment (n = 35), dementia of the Alzheimer's type (n = 12), other dementias or neurodegenerative diseases (n = 41), or were cognitively normal controls (n = 6). We determined the cutoffs of CSF biomarkers that optimized their agreement with 18F-Florbetapir PET. The cutoff selection for single biomarkers was based on clear Youden peaks indicating optimal sensitivity and specificity at 916 pg/mL for Aβ42, 456 pg/mL for t-tau, and 63 pg/mL for p-tau. For biomarker ratios, the best cutoffs were: 0.062 for Aβ42/Aβ40, 0.62 for t-tau/Aβ42, and 0.068 for p-tau/Aβ42.[54]

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CSF samples were collected following international consensus recommendations.[55] Regarding the PGA project, biomarker analyses for AD were performed using a single-analyte enzyme-linked immunosorbent assay (ELISA; INNOTEST, Fujirebio Europe, Ghent, Belgium). The cutoffs for core AD biomarkers were based on those described by Alcolea et al, 2015.[56] with a 5% confidence interval added to the original cutoff for Aβ42, following the method outlined by Landau et al., 2013.[57] The defined cutoffs were: 580 pg/mL for Aβ42, 350 pg/mL for t-tau, and 61 pg/mL for p-tau. As for the DEBA project, CSF analyses were conducted using Elecsys® assays for Aβ42, t-tau and p-tau. The following cutoffs were applied: 1030 pg/mL for Aβ42, 300 pg/mL for t-tau, 27 pg/mL for p-tau, 0.28 for the t-tau/Aβ42 ratio, and 0.023 for the p-tau/Aβ42 ratio.

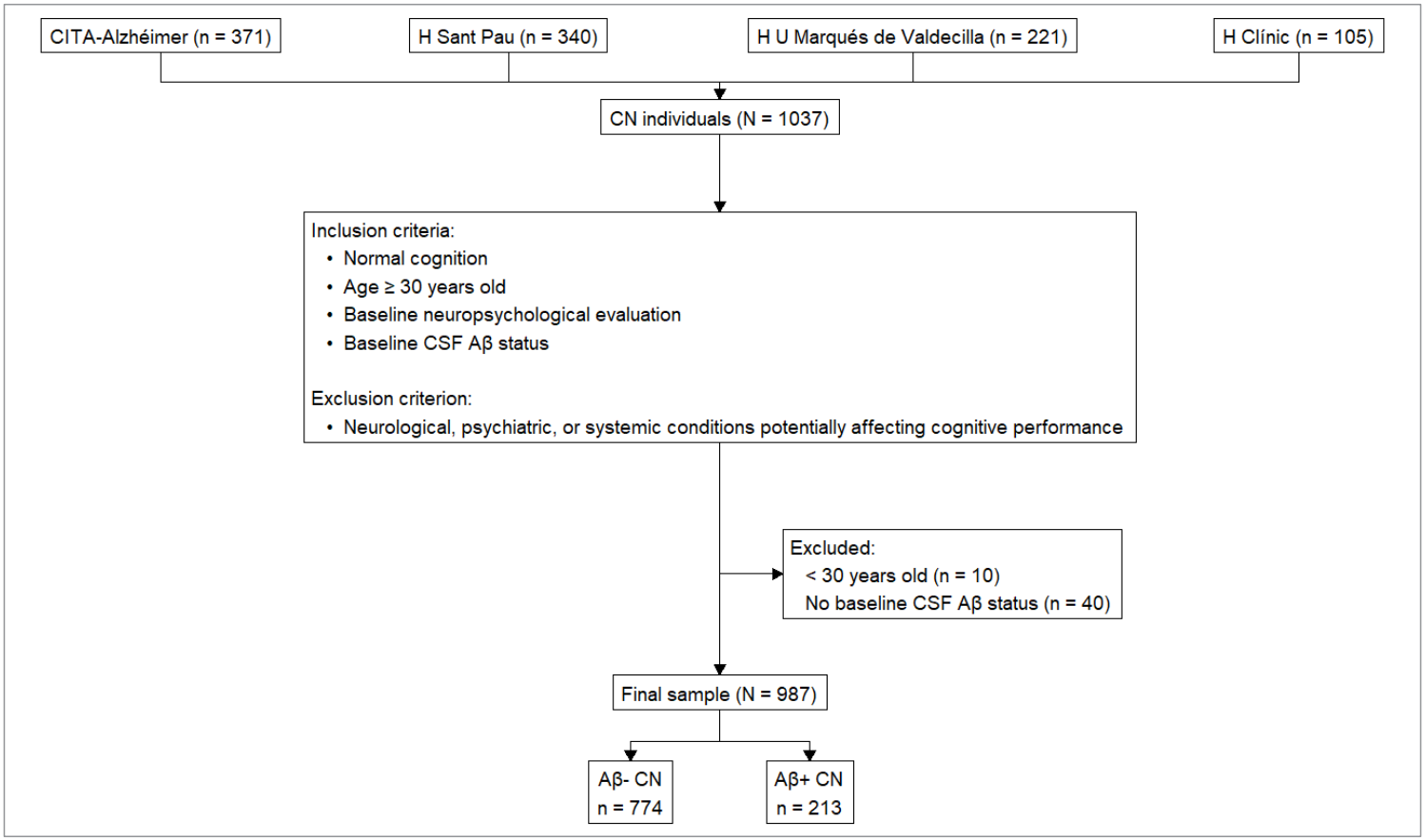
Hospital Clínic de Barcelona

All subjects underwent a lumbar puncture between 9 a.m. and 12 p.m. 10 mL of CSF were centrifuged at 2000 g for 10 minutes at 4ºC within the first hour after collection, and stored at -80ºC in polypropylene tubes until usage. CSF Aβ42, t-tau, and p-tau levels were measured by INNOTEST Enzyme-Linked ImmunoSorbent Assays (ELISAs) following the manufacturer's instructions (Fujirebio, Ghent, Belgium). The following cutoff points were considered: 550 pg/mL (for CSF samples analyzed before February 2016) and 750 pg/mL (for CSF samples analyzed after February 2016) for Aβ42, 385 pg/mL for t-tau, and 65 pg/mL for p-tau.

Hospital Universitario Marqués de Valdecilla

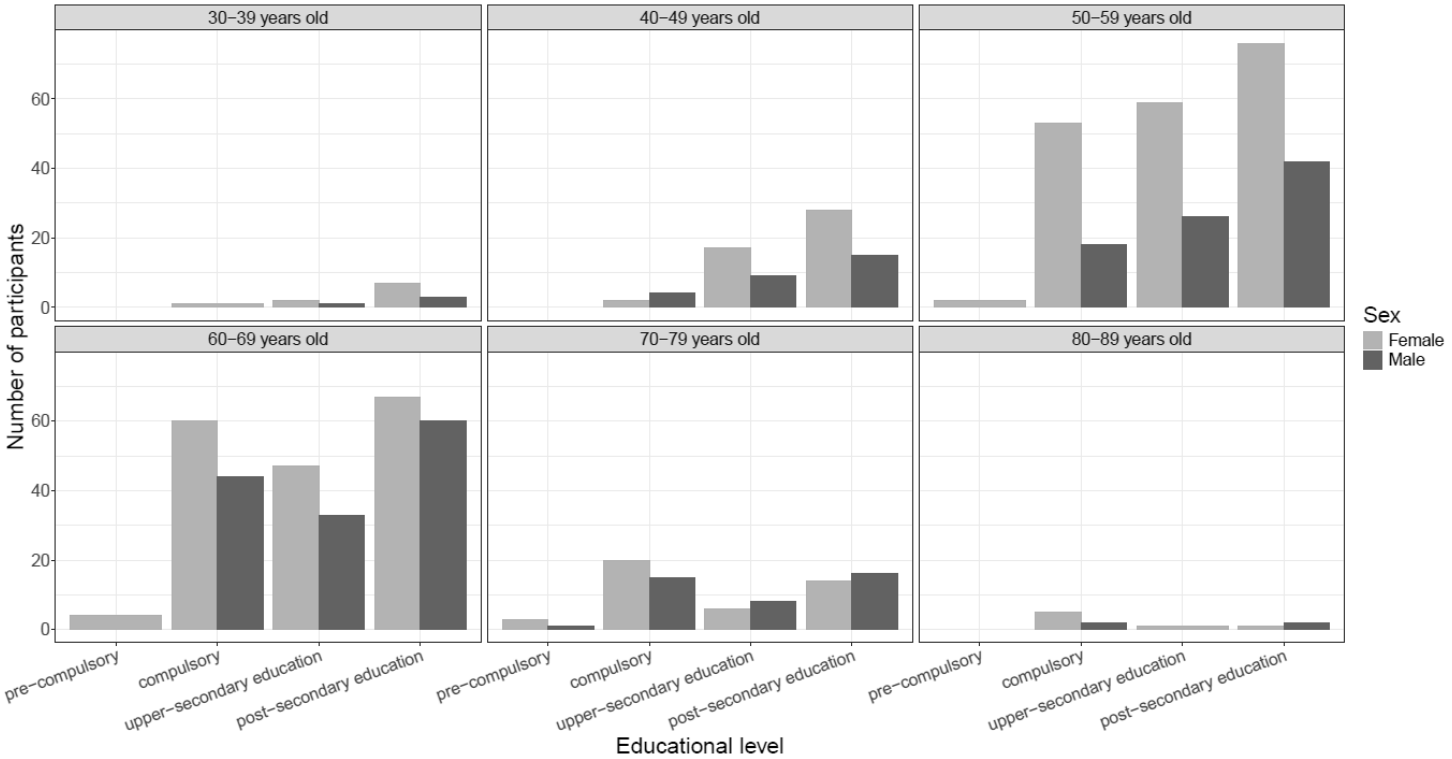
CSF was collected and stored according to international consensus recommendations. The lumbar puncture was performed with the subject fasting, using a standard 22 G needle, in lateral decubitus, between the L3-L5 spaces. CSF samples were collected in 15 mL polypropylene tubes and centrifuged at 2000 g for 10 minutes at room temperature. The resultant was aliquoted in volumes of 500 µL into 1 mL tubes and frozen at -80 °C until analysis in our hospital's immunology laboratory. The evaluation of CSF AD biomarkers included the determination of Aβ42, Aβ40, t-tau, and p-tau. Biomarker levels were quantified by chemiluminescent enzyme immunoassay (Lumipulse G600 II, Fujirebio Europe, Belgium) following the manufacturer's instructions. Cutoff levels were calculated for our cohort using Gaussian Mixture Models: 0.076 for the Aβ42/40 ratio, 543 pg/mL for t-tau, and 73.2 pg/mL for p-tau.

Supplementary Figure 1. Flowchart of participant recruitment and sample composition



Abbreviations: Aβ, amyloid β; Aβ−, Aβ-negative; Aβ+, Aβ-positive; CN, cognitively normal; CSF, cerebrospinal fluid.

Supplementary Figure 2. Representativeness of the normative dataset



Stratification of the normative dataset (n = 774) by age decade, educational level [according to the International Standard Classification of Education (ISCED) 2011 for Spain, as developed by the United Nations Educational, Scientific and Cultural Organization (UNESCO)[58]], and sex to assess its representativeness.

Supplementary Table 1. Criteria and measures used to classify CN individuals

H. Sant Pau	CITA-Alzhéimer	H. Clínic	H.U. Marqués Valdecilla
Definition of CN			
All must be met: · Cognitive performance within the normal range (defined as achieving scaled scores ≥7, scores ≥−1.5 SD from the normative mean, or scores exceeding established cutoff values for impairment, depending on the normative data available for each test) on all neuropsychological tests. · CDR global score of 0.			
Neuropsychological battery			
Screening tests and scales			
• **MMSE**[21] • GDS[59] • NPI[60] • IDDD[61] • GDS-FAST[62] • **CDR**[63]	• **MMSE**[21] • T@M[64] • HAD[65] • PSQ[66] • WAIS-III – Vocabulary subtest[67] • **CDR**[63]	• **MMSE**[21] • GDS-FAST[62] • **CDR**[63]	• **MMSE**[21] • GDS[59] • IPAQ[68] • COS[69] • MFE-30[70] • CRQ[71] • IDDD[61] • **CDR**[63]
Visuospatial skills			
• **VOSP number location**[24,25] • **ROCF copy**[26,27] • CERAD figure copy[72] • Poppelreuter-Ghent's Overlapping Figures Test[73] • Clock drawing test – copy[74]	• **VOSP number location***[24,25] • VOSP object decision*[24,25] • VOSP Judgement of Line Orientation†[24,25] • **ROCF copy**[26,27]	• **VOSP number location**[24,25] • **ROCF copy**[26,27]	• **VOSP number location**[24,25] • **ROCF copy**[26,27] • CERAD figure copy[72] • Poppelreuter-Ghent's Overlapping Figures Test[73]
Memory			
• **FCSRT total free recall**[26,27] • **FCSRT total recall**[26,27] • **FCSRT delayed free recall**[26,27] • **FCSRT delayed total recall**[26,27] • CERAD Word List Memory Test[72] • WMS-IV – Logical Memory[75] • **ROCF delayed recall**[26,27] • CERAD figure delayed recall[76]	• **FCSRT total free recall**†[26,27] • **FCSRT total recall**†[26,27] • **FCSRT delayed free recall**†[26,27] • **FCSRT delayed total recall**†[26,27] • CERAD Word List Memory Test*[72] • **ROCF delayed recall**[26,27]	• **FCSRT total free recall**[26,27] • **FCSRT total recall**[26,27] • **FCSRT delayed free recall**[26,27] • **FCSRT delayed total recall**[26,27] • CERAD Word List Memory Test[72] • **ROCF delayed recall**[26,27]	• **FCSRT total free recall**[26,27] • **FCSRT total recall**[26,27] • **FCSRT delayed free recall**[26,27] • **FCSRT delayed total recall**[26,27] • WMS-IV – Logical Memory[75] • **ROCF delayed recall**[26,27] • CERAD figure delayed recall[76]
Attention/executive functioning			
• **TMT-A**[28,29] • **TMT-B**[28,29] • **Digit Span Forward**[28,29] • **Digit Span Backward**[28,29] • Clock drawing test – command[74] • **Phonetic fluency**[30,31]	• **TMT-A**[28,29] • **TMT-B**[28,29] • **Digit Span Forward**[28,29] • **Digit Span Backward**[28,29] • **Phonetic fluency**[30,31]	• **TMT-A**[28,29] • **TMT-B**[28,29] • **Digit Span Forward**[28,29] • **Digit Span Backward**[28,29] • Clock drawing test – command[74] • **Phonetic fluency**[30,31]	• **TMT-A**[28,29] • **TMT-B**[28,29] • **Digit Span Forward**[28,29] • **Digit Span Backward**[28,29] • WAIS-IV – Coding subtest[77] • **Phonetic fluency**[30,31]
Language			
• **Semantic fluency**[30,31] • **BNT**[32–34]	• **Semantic fluency**[30,31] • **BNT**[32–34]	• **Semantic fluency**[30,31] • **BNT**[32–34]	• **Semantic fluency**[30,31] • **BNT**[32–34]

Criteria and neuropsychological test batteries used to classify CN individuals at each participating center [i.e., Hospital de la Santa Creu i Sant Pau (Barcelona), Fundación CITA-Alzhéimer (San Sebastián), Hospital Clínic de Barcelona (Barcelona), and Hospital Universitario Marqués de Valdecilla (Santander)]. Measures common to all centers' protocols are in bold. The CITA-Alzhéimer Foundation established two cohorts: DEBA (a cross-sectional epidemiological study) and GAP (a longitudinal study); subtle between-cohort differences in the neuropsychological protocols are indicated by the following symbols:

* Indicates tests administered exclusively in the DEBA cohort.

† Indicates tests administered exclusively in the GAP cohort.

References [21,24–34] cited in this supplementary table are included in the main manuscript's reference list.

Abbreviations: BNT, Boston Naming Test; CDR, Clinical Dementia Rating; CERAD, Consortium to Establish a Registry for Alzheimer's Disease; CN, cognitively normal; COS, Cuestionario Oviedo de Sueño (Oviedo Sleep Questionnaire); CRQ, Cognitive Reserve Questionnaire; FCSRT, Free and Cued Selective Reminding Test; GDS, Geriatric Depression Scale; GDS-FAST, the combined Global Deterioration Scale and Functional Assessment Staging Tool; HAD, Hospital Anxiety and Depression Scale; IDDD, Interview for Deterioration in Daily Living in Dementia; IPAQ, International Physical Activity Questionnaire; MFE-30, Memory Failures in Everyday; MMSE, Mini-Mental State Examination; NPI, Neuropsychiatric Inventory; PSQ, Perceived Stress Questionnaire; ROCF, Rey-Osterrieth Complex Figure; SD, standard deviation; T@M, Test de Alteración de Memoria (Memory Alteration Test); TMT-A/B, Trail Making Test Part A/Part B; VOSP, Visual Object and Space Perception Battery; WAIS-III, Wechsler Adult Intelligence Scale–Third Edition; WAIS-IV, Wechsler Adult Intelligence Scale–Fourth Edition; WMS–IV, Wechsler Memory Scale–Fourth Edition.

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11.2. Supplementary material for article 2: Amyloid-negative neuropsychological norms: Added value in the era of biomarkers and disease-modifying therapies

Supplementary Methods

Cerebrospinal fluid (CSF) biomarker analyses by center

Hospital de la Santa Creu i Sant Pau

CSF was collected and processed in polypropylene tubes following international recommendations.[44,45] The first 2 mL of CSF were transferred to the general laboratory for cell count, and glucose and protein level analyses. Another 15-20 mL volume was transferred to our laboratory, where samples were processed and aliquoted within the first 2 hours after the lumbar puncture. Aliquots were stored at −80ºC until analysis. Core Alzheimer's disease (AD) biomarkers [amyloid β 1-42 (Aβ42), amyloid β 1-40 (Aβ40), total tau (t-tau), and phosphorylated tau at threonine-181 (p-tau)] were measured in all participants. Biomarker cutoffs were derived from 96 participants of the Sant Pau Initiative on Neurodegeneration (SPIN cohort)[46] with available 18F-Florbetapir positron emission tomography (PET) using the Lumipulse G System. Participants had mild cognitive impairment (n = 35), dementia of the Alzheimer's type (n = 12), other dementias or neurodegenerative diseases (n = 41), or were cognitively normal controls (n = 6). We determined the cutoffs of CSF biomarkers that optimized their agreement with 18F-Florbetapir PET. The cutoff selection for single biomarkers was based on clear Youden peaks indicating optimal sensitivity and specificity at 916 pg/mL for Aβ42, 456 pg/mL for t-tau, and 63 pg/mL for p-tau. For biomarker ratios, the best cutoffs were: 0.062 for Aβ42/Aβ40, 0.62 for t-tau/Aβ42, and 0.068 for p-tau/Aβ42.[47]

Fundación CITA-Alzhéimer

CSF samples were collected following international consensus recommendations.[48] Regarding the PGA project, biomarker analyses for AD were performed using a single-analyte enzyme-linked immunosorbent assay (ELISA; INNOTEST, Fujirebio Europe, Ghent, Belgium). The cutoffs for core AD biomarkers were based on those described by Alcolea et al, 2015.[49] with a 5% confidence interval added to the original cutoff for Aβ42, following the method outlined by Landau et al., 2013.[50] The defined cutoffs were: 580 pg/mL for Aβ42, 350 pg/mL for t-tau, and 61 pg/mL for p-tau. As for the DEBA project, CSF analyses were conducted using Elecsys® assays for Aβ42, t-tau and p-tau. The following cutoffs were applied: 1030 pg/mL for Aβ42, 300 pg/mL for t-tau, 27 pg/mL for p-tau, 0.28 for the t-tau/Aβ42 ratio, and 0.023 for the p-tau/Aβ42 ratio.

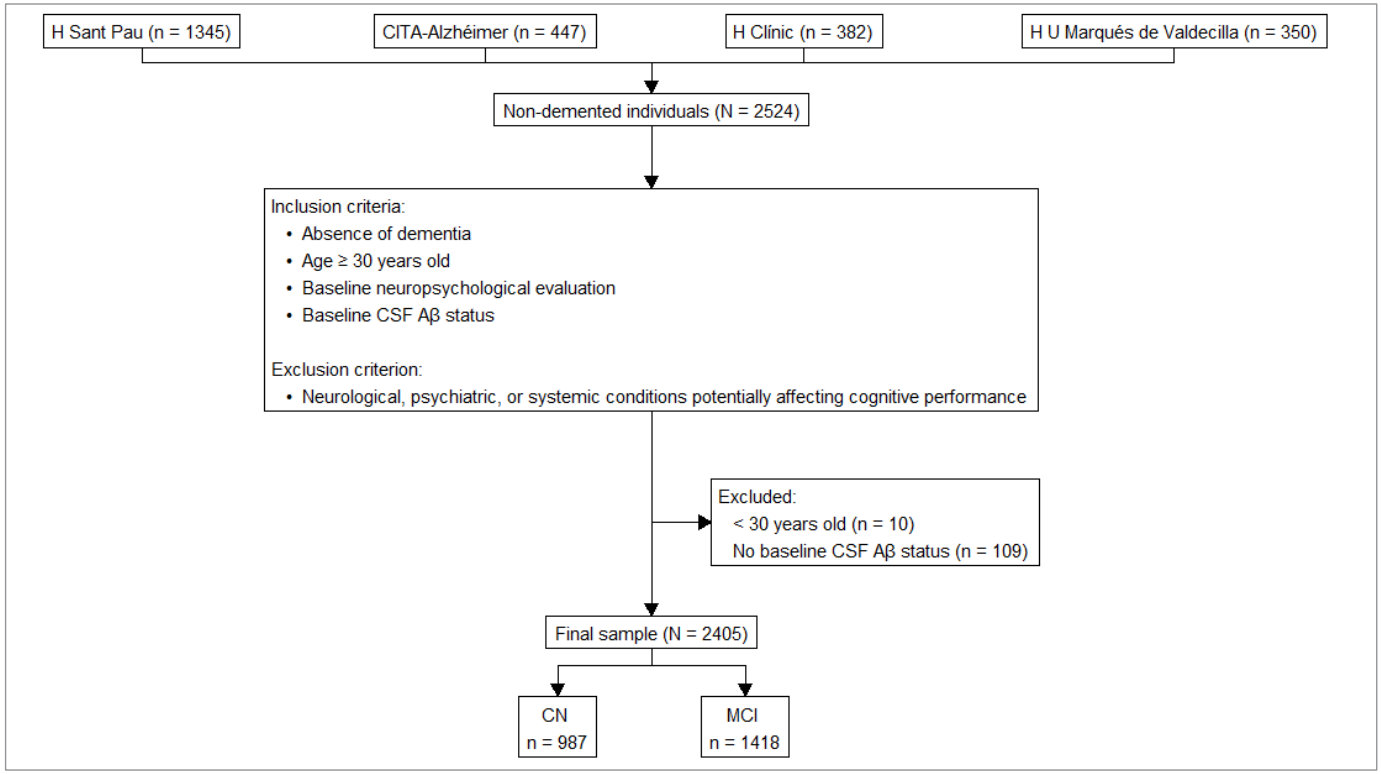
Hospital Clínic de Barcelona

All subjects underwent a lumbar puncture between 9 a.m. and 12 p.m. 10 mL of CSF were centrifuged at 2000 g for 10 minutes at 4ºC within the first hour after collection, and stored at -80ºC in polypropylene tubes until usage. CSF Aβ42, t-tau, and p-tau levels were measured by INNOTEST Enzyme-Linked ImmunoSorbent Assays (ELISAs) following the manufacturer's instructions (Fujirebio, Ghent, Belgium). The following cutoff points were considered: 550 pg/mL (for CSF samples analyzed before February 2016) and 750 pg/mL (for CSF samples analyzed after February 2016) for Aβ42, 385 pg/mL for t-tau, and 65 pg/mL for p-tau.

Hospital Universitario Marqués de Valdecilla

CSF was collected and stored according to international consensus recommendations. The lumbar puncture was performed with the subject fasting, using a standard 22 G needle, in lateral decubitus, between the L3-L5 spaces. CSF samples were collected in 15 mL polypropylene tubes and centrifuged at 2000 g for 10 minutes at room temperature. The resultant was aliquoted in volumes of 500 µL into 1 mL tubes and frozen at -80 °C until analysis in our hospital's immunology laboratory. The evaluation of CSF AD biomarkers included the determination of Aβ42, Aβ40, t-tau, and p-tau. Biomarker levels were quantified by chemiluminescent enzyme immunoassay (Lumipulse G600 II, Fujirebio Europe, Belgium) following the manufacturer's instructions. Cutoff levels were calculated for our cohort using Gaussian Mixture Models: 0.076 for the Aβ42/40 ratio, 543 pg/mL for t-tau, and 73.2 pg/mL for p-tau.

Supplementary Figure 1. Flowchart of participant recruitment and sample composition



Abbreviations: Aβ, amyloid β; CN, cognitively normal; CSF, cerebrospinal fluid; MCI, mild cognitive impairment.

Supplementary Table 1. Neuropsychological evaluation

H. Sant Pau	CITA-Alzhéimer	H. Clínic	H.U. Marqués Valdecilla
Neuropsychological battery			
Screening tests and scales			
• **MMSE**[21] • GDS[51] • NPI[52] • IDDD[53] • GDS-FAST[54] • **CDR**[55]	• **MMSE**[21] • T@M[56] • HAD[57] • PSQ[58] • WAIS-III – Vocabulary subtest[59] • **CDR**[55]	• **MMSE**[21] • GDS-FAST[54] • **CDR**[55]	• **MMSE**[21] • GDS[51] • IPAQ[60] • COS[61] • MFE-30[62] • CRQ[63] • IDDD[53] • **CDR**[55]
Visuospatial skills			
• **VOSP number location**[64,65] • **ROCF copy**[66,67] • CERAD figure copy[68] • Poppelreuter-Ghent's Overlapping Figures Test[69] • Clock drawing test – copy[70]	• **VOSP number location***[64,65] • VOSP object decision*[64,65] • VOSP Judgement of Line Orientation†[64,65] • **ROCF copy**[66,67]	• **VOSP number location**[64,65] • **ROCF copy**[66,67]	• **VOSP number location**[64,65] • **ROCF copy**[66,67] • CERAD figure copy[68] • Poppelreuter-Ghent's Overlapping Figures Test[69]
Memory			
• **FCSRT total free recall**[66,67] • **FCSRT total recall**[66,67] • **FCSRT delayed free recall**[66,67] • **FCSRT delayed total recall**[66,67] • CERAD Word List Memory Test[68] • WMS-IV – Logical Memory[71] • **ROCF delayed recall**[66,67] • CERAD figure delayed recall[72]	• **FCSRT total free recall**†[66,67] • **FCSRT total recall**†[66,67] • **FCSRT delayed free recall**†[66,67] • **FCSRT delayed total recall**†[66,67] • CERAD Word List Memory Test*[68] • **ROCF delayed recall**[66,67]	• **FCSRT total free recall**[66,67] • **FCSRT total recall**[66,67] • **FCSRT delayed free recall**[66,67] • **FCSRT delayed total recall**[66,67] • CERAD Word List Memory Test[68] • **ROCF delayed recall**[66,67]	• **FCSRT total free recall**[66,67] • **FCSRT total recall**[66,67] • **FCSRT delayed free recall**[66,67] • **FCSRT delayed total recall**[66,67] • WMS-IV – Logical Memory[71] • **ROCF delayed recall**[66,67] • CERAD figure delayed recall[72]
Attention/executive functioning			
• **TMT-A**[73,74] • **TMT-B**[73,74] • **Digit Span Forward**[73,74] • **Digit Span Backward**[73,74] • Clock drawing test – command[70] • **Phonetic fluency**[75,76]	• **TMT-A**[73,74] • **TMT-B**[73,74] • **Digit Span Forward**[73,74] • **Digit Span Backward**[73,74] • **Phonetic fluency**[75,76]	• **TMT-A**[73,74] • **TMT-B**[73,74] • **Digit Span Forward**[73,74] • **Digit Span Backward**[73,74] • Clock drawing test – command[70] • **Phonetic fluency**[75,76]	• **TMT-A**[73,74] • **TMT-B**[73,74] • **Digit Span Forward**[73,74] • **Digit Span Backward**[73,74] • WAIS-IV – Coding subtest[77] • **Phonetic fluency**[75,76]
Language			
• **Semantic fluency**[75,76] • **BNT**[78–80]	• **Semantic fluency**[75,76] • **BNT**[78–80]	• **Semantic fluency**[75,76] • **BNT**[78–80]	• **Semantic fluency**[75,76] • **BNT**[78–80]

Neuropsychological evaluation protocols across the participating centers [i.e., Hospital de la Santa Creu i Sant Pau (Barcelona), Fundación CITA-Alzhéimer (San Sebastián), Hospital Clínic de Barcelona (Barcelona), and Hospital Universitario Marqués de Valdecilla (Santander)]. Measures common to all centers' protocols are in bold. The CITA-Alzhéimer Foundation established two cohorts: DEBA (a

cross-sectional epidemiological study) and GAP (a longitudinal study); subtle between-cohort differences in the neuropsychological protocols are indicated by the following symbols:
* Indicates tests administered exclusively in the DEBA cohort.
† Indicates tests administered exclusively in the GAP cohort.
Reference [21] cited in this supplementary table is included in the main manuscript's reference list.

Abbreviations: BNT, Boston Naming Test; CDR, Clinical Dementia Rating; CERAD, Consortium to Establish a Registry for Alzheimer's Disease; COS, Cuestionario Oviedo de Sueño (Oviedo Sleep Questionnaire); CRQ, Cognitive Reserve Questionnaire; FCSRT, Free and Cued Selective Reminding Test; GDS, Geriatric Depression Scale; GDS-FAST, the combined Global Deterioration Scale and Functional Assessment Staging Tool; HAD, Hospital Anxiety and Depression Scale; IDDD, Interview for Deterioration in Daily Living in Dementia; IPAQ, International Physical Activity Questionnaire; MFE-30, Memory Failures in Everyday; MMSE, Mini-Mental State Examination; NPI, Neuropsychiatric Inventory; PSQ, Perceived Stress Questionnaire; ROCF, Rey-Osterrieth Complex Figure; T@M, Test de Alteración de Memoria (Memory Alteration Test); TMT-A/B, Trail Making Test Part A/Part B; VOSP, Visual Object and Space Perception Battery; WAIS-III, Wechsler Adult Intelligence Scale–Third Edition; WAIS-IV, Wechsler Adult Intelligence Scale–Fourth Edition; WMS–IV, Wechsler Memory Scale–Fourth Edition.

Supplementary Table 2. Comparison of TN-derived actuarial classifications versus conventional diagnoses

	TN-derived actuarial diagnoses		Total
Conventional diagnoses	CN	MCI	
CN	790 (95.5%)	37 (4.5%)	827 (100.0%)
MCI	524 (38.4%)	842 (61.6%)	1366 (100.0%)

Of the 2405 participants, 2193 had enough baseline neuropsychological data to receive an actuarial cognitive classification based on TN.

Abbreviations: CN, cognitively normal; MCI, mild cognitive impairment; TN, traditional norms.

Supplementary Table 3. Comparison of NGN-derived actuarial classifications versus conventional diagnoses

	NGN-derived actuarial diagnoses		Total
Conventional diagnoses	**CN**	**MCI**	
CN	760 (91.6%)	70 (8.4%)	830 (100.0%)
MCI	329 (24.0%)	1044 (76.0%)	1373 (100.0%)

Of the 2405 participants, 2203 had enough baseline neuropsychological data to receive an actuarial cognitive classification based on NGN.

Abbreviations: CN, cognitively normal; MCI, mild cognitive impairment; NGN, next-generation norms.

Supplementary Table 4. Characteristics of the longitudinal dataset

Characteristics	Overall N = 812*	CN_{tn}/CN_{ngn} n = 374*	MCI_{tn}/CN_{ngn} n = 8*	CN_{tn}/MCI_{ngn} n = 105*	MCI_{tn}/MCI_{ngn} n = 325*	*P* value†	Effect size‡
Age (years)	68 (10.4)	64 (11.1)	54 (11.3)	72 (8.4)	71 (8.6)	**< .001**	$\eta^2 = 0.12$
Education (years)	12 (5.1)	13 (5.2)	13 (6.3)	10 (4.7)	12 (4.9)	**< .001**	$\eta^2 = 0.03$
Biological sex (female)	455 (56%)	219 (59%)	5 (63%)	63 (60%)	168 (52%)	.2	φc = 0.04
CSF Aβ status						**< .001**	φc = 0.38
Aβ+	322 (40%)	75 (20%)	1 (13%)	56 (53%)	190 (58%)		
Aβ−	490 (60%)	299 (80%)	7 (88%)	49 (47%)	135 (42%)		
MMSE at first visit	27.1 (2.7)	28.2 (1.9)	27.8 (3.7)	26.5 (2.8)	25.9 (2.9)	**< .001**	$\eta^2 = 0.17$
Number of visits						.2	$\eta^2 = 0.01$
Mean (SD)	3.6 (1.9)	3.7 (1.9)	3.0 (2.1)	3.3 (1.7)	3.5 (1.9)		
Median (range)	3.0 (2-11)	3.0 (2-11)	2.0 (2-8)	3.0 (2-10)	3.0 (2-11)		
Follow-up time						.2	$\eta^2 = 0.01$
Mean (SD)	4.2 (2.7)	4.4 (2.7)	3.2 (2.3)	4.2 (2.7)	4.0 (2.5)		
Median (range)	3.5 (1.0-14.1)	3.8 (1.0-14.1)	1.9 (1.4-7.7)	3.3 (1.0-12.3)	3.5 (1.0-12.9)		

Descriptive statistics and inferential analyses for the longitudinal dataset, including demographics, CSF Aβ status and MMSE scores at baseline, and the number and duration of follow-up visits. Results are presented for the complete longitudinal dataset and stratified by actuarially reclassified diagnostic groups.

* Mean (SD); median (range); n (%)

† One-way ANOVA; Welch's ANOVA; Pearson's chi-squared test. Bold *P* values indicate statistical significance (< .05).

‡ η^2= eta-squared, φc= Cramér's V

Abbreviations: Aβ, amyloid β; Aβ−, Aβ-negative; Aβ+, Aβ-positive; CN, cognitively normal; CSF, cerebrospinal fluid; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; subscripts "tn" and "ngn" refer to actuarial cognitive classification by traditional norms and next-generation norms, respectively.

Supplementary Table 5. Temporal changes in MMSE scores for the complete longitudinal dataset: LME model results

Term	MMSE change estimate (95% CI)	*P* value
Intercept	31.11 (29.93 to 32.28)	**<.001**
Age	−0.07 (−0.09 to −0.06)	**<.001**
Education	0.15 (0.12 to 0.18)	**<.001**
Sex (male)	0.33 (0.05 to 0.61)	**<.05**
Time	−0.1 (−0.19 to −0.01)	**<.05**
CN_{tn}/MCI_{ngn}	−0.72 (−1.16 to −0.28)	**<.01**
MCI_{tn}/MCI_{ngn}	−1.97 (−2.28 to −1.67)	**<.001**
Time:CN_{tn}/MCI_{ngn}	−0.32 (−0.52 to −0.12)	**<.01**
Time:MCI_{tn}/MCI_{ngn}	−0.8 (−0.94 to −0.66)	**<.001**

LME-model estimates of temporal changes in MMSE scores for the complete longitudinal dataset (N = 812) with 95% CI. The model includes diagnostic groups (reference group: CN_{tn}/CN_{ngn}), time, their interaction, and demographic covariates [age, education, and sex (reference category: male)]. Bold *P* values indicate statistical significance (< .05).

Abbreviations: CI, confidence interval; CN, cognitively normal; LME, linear mixed-effects; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; subscripts "tn" and "ngn" refer to actuarial cognitive classification by traditional norms and next-generation norms, respectively.

Supplementary Table 6. Temporal changes in MMSE scores for the Aβ+ longitudinal dataset: LME model results

Term	MMSE change estimate (95% CI)	*P* value
Intercept	30.38 (27.35 to 33.42)	**<.001**
Age	−0.07 (−0.11 to −0.03)	**<.01**
Education	0.18 (0.12 to 0.24)	**<.001**
Sex (male)	0.63 (0.09 to 1.17)	**<.05**
Time	−0.36 (−0.63 to −0.09)	**<.01**
CN_{tn}/MCI_{ngn}	−0.97 (−1.78 to −0.15)	**<.05**
MCI_{tn}/MCI_{ngn}	−2.46 (−3.08 to −1.83)	**<.001**
Time:CN_{tn}/MCI_{ngn}	−0.26 (−0.67 to 0.15)	0.22
Time:MCI_{tn}/MCI_{ngn}	−0.88 (−1.2 to −0.56)	**<.001**

LME-model estimates of temporal changes in MMSE scores for the Aβ+ longitudinal dataset (n = 322), with 95% CI. The model includes diagnostic groups (reference group: CN_{tn}/CN_{ngn}), time, their interaction, and demographic covariates [age, education, and sex (reference category: male)]. Bold *P* values indicate statistical significance (< .05).

Abbreviations: Aβ+, amyloid β-positive; CI, confidence interval; CN, cognitively normal; LME, linear mixed-effects; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; subscripts "tn" and "ngn" refer to actuarial cognitive classification by traditional norms and next-generation norms, respectively.

Supplementary Table 7. Temporal changes in MMSE scores for the Aβ− longitudinal dataset: LME model results

Term	MMSE change estimate (95% CI)	*P* value
Intercept	31.41 (30.34 to 32.49)	**<.001**
Age	−0.07 (−0.09 to −0.06)	**<.001**
Education	0.12 (0.09 to 0.15)	**<.001**
Sex (male)	−0.04 (−0.32 to 0.25)	0.802
Time	−0.03 (−0.09 to 0.02)	0.213
CN_{tn}/MCI_{ngn}	−0.52 (−0.98 to −0.05)	**<.05**
MCI_{tn}/MCI_{ngn}	−1.46 (−1.78 to −1.14)	**<.001**
Time:CN_{tn}/MCI_{ngn}	−0.08 (−0.22 to 0.06)	0.286
Time:MCI_{tn}/MCI_{ngn}	−0.33 (−0.42 to −0.24)	**<.001**

LME-model estimates of temporal changes in MMSE scores for the Aβ− longitudinal dataset (n = 490), with 95% CI. The model includes diagnostic groups (reference group: CN_{tn}/CN_{ngn}), time, their interaction, and demographic covariates [age, education, and sex (reference category: male)]. Bold *P* values indicate statistical significance (< .05).

Abbreviations: Aβ−, amyloid β-negative; CI, confidence interval; CN, cognitively normal; LME, linear mixed-effects; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; subscripts "tn" and "ngn" refer to actuarial cognitive classification by traditional norms and next-generation norms, respectively.

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