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Neuropsychological and fluid biomarker changes in the Alzheimer's disease continuum in adults with Down syndrome

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Doctoral Thesis

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CERTIFICATE of DIRECTION

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Certify:

That the work "**Neuropsychological and fluid biomarker changes in the Alzheimer's disease continuum in adults with Down syndrome**", presented by Laura Videla Toro to qualify for Doctor in Neurociènces for the Universitat Autònoma de Barcelona has been done under our direction and meets all the requirements to be presented and defended in the presence of the corresponding Thesis Committee.

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ABSTRACT

Down syndrome (DS) is the most common cause of intellectual disability (ID) of genetic origin, and it is caused by the triplication of the chromosome 21. Some genes coded in this chromosome such as the amyloid precursor protein (APP), lead to the universal presence of Alzheimer's disease (AD) pathological hallmarks in adults with DS by the age of 40, and, subsequently, to an ultra-high risk to develop AD dementia. However, the clinical diagnosis of dementia in individuals with DS is a challenge due to the associated neurodevelopmental ID, and the lack of validated clinical criteria and neuropsychological normative data to diagnose symptomatic AD. Currently, there are no treatments to prevent or stop AD in this population, and adults with DS have historically been excluded from AD clinical trials and research denying them the opportunity to benefit from potential treatments and scientific breakthroughs.

This doctoral thesis aimed to better characterize the clinical and cognitive course of AD in this population, and to study the performance of different fluid biomarkers in plasma and cerebrospinal fluid (CSF). Our data is based on the *Down Alzheimer Barcelona Neuroimaging Initiative* (DABNI) cohort of adults with DS, the largest cohort for the study of AD in DS worldwide.

Our works have shown the nearly inevitable progression to symptomatic AD with age from the fourth decade of life in this population, highlighting the urgent need for the design of health plans and clinical trials against AD in individuals with DS. In this sense, a prerequisite is to validate neuropsychological tools and define clinical diagnostic criteria for AD in DS. In contrast with previous assumptions, we demonstrate that cognitive assessment is feasible in adults with DS, and that AD-related cognitive decline can be detected with excellent accuracy in single-points evaluations. Moreover, we also showed the feasibility of performing longitudinal cognitive assessments for the duration of preventive clinical trials and validate the performance of two commonly used neuropsychological tools to be used as cognitive endpoints, which are essential in AD clinical trials. Finally, biomarkers have revolutionized the diagnosis of AD in the general population. These advances must be adapted and applied in DS. In this regard, we have validated the diagnostic and prognostic performance of plasma NfL and

have explored a panel of synaptic proteins in CSF as novel biomarkers for AD in DS that may be useful to monitor therapeutic response in AD clinical trials.

In brief, the data presented in this thesis has focused on the diagnosis of symptomatic AD and the study of clinical, cognitive, and biomarker changes of AD in adults with DS. These multimodal approaches are essential to provide new insights to the natural history of AD, to establish accurate diagnostic tools, and, potentially, to discover new therapeutic targets.

RESUM

La síndrome de Down (SD) és la causa més freqüent de discapacitat intel·lectual (DI) d'origen genètic i està causada per la triplicació del cromosoma 21. Alguns dels gens codificats en aquest cromosoma, principalment el de la proteïna precursora de l'amiloide (APP), fan que aquestes persones presentin les marques fisiopatològics de la malaltia d'Alzheimer (MA) a partir dels 40 anys i que, per tant, tinguin un elevadíssim risc de desenvolupar una demència per MA. No obstant, el diagnòstic clínic de la demència en aquesta població és un repte degut a la DI premòrbida associada a la pròpia SD, a la manca de criteris diagnòstics adaptats, i a la manca de dades normatives dels tests neuropsicològics. Actualment no hi ha tractaments per prevenir o curar la MA i, a més, les persones amb SD han estat històricament excloses dels assaigs clínics i la recerca, negant-los l'oportunitat de beneficiar-se de possibles tractaments i avenços científics.

L'objectiu d'aquesta tesi doctoral es centra en la caracterització del curs clínic i cognitiu de la MA en aquesta població i en l'estudi del rendiment diagnòstic i pronòstic de diferents biomarcadors de fluids en plasma i líquid cefaloraquidi (LCR). Per fer-ho, ens hem basat en dades de la cohort més gran d'arreu del món d'adults amb la SD per l'estudi de la MA, la cohort *Down Alzheimer Barcelona Neuroimaging Initiative* (DABNI).

Concretament, descrivim com entre la població adulta amb la SD és quasi inevitable la progressió cap a la fase de demència de la MA a mida que envelleixen i, especialment, a partir de la quinta dècada de vida. Així, posem de manifest la urgent necessitat de dissenyar plans de salut i assaigs clínics específics contra la MA per aquesta població. D'aquesta manera, serà imprescindible validar eines neuropsicològiques i definir uns criteris diagnòstics adaptats a persones amb la SD. En contra del que s'ha assumit fins al moment en el camp de la DI, i de la síndrome de Down en particular, en la present tesi demostrem que és factible avaluar el deteriorament cognitiu de la MA amb una avaluació transversal. A més, mostrem la viabilitat de monitoritzar el rendiment cognitiu de les persones amb la SD al llarg d'un assaig clínic preventiu validant dues eines neuropsicològiques com a variables d'eficàcia (*endpoints*) cognitives, imprescindibles en qualsevol assaig clínic per la MA. Finalment, en un context on els biomarcadors han revolucionat la manera de diagnosticar la MA en la població general,

destaquem la necessitat d'adaptar aquests avenços per aplicar-los a la població amb la SD. En aquest sentit, hem validat el rendiment diagnòstic i pronòstic dels neurofilaments de cadena lleugera (NfL) en plasma per la detecció de la MA en la SD i hem explorat un panell de proteïnes sinàptiques en líquid cefaloraquidi (LCR) com a possibles biomarcadors de la MA que podrien ser útils per monitoritzar la resposta terapèutica assaigs clínics.

En resum, les dades presentades en aquesta tesi doctoral s'han centrat en el diagnòstic de les fases simptomàtiques de la MA i en l'estudi dels canvis clínics, cognitius i de biomarcadors de la malaltia en adults amb la SD. Estudis multimodals com els presentats en aquesta recull són essencials per avançar en el coneixement de la història natural de la MA, per desenvolupar noves eines diagnòstiques precises i, potencialment, per descobrir noves dianes terapèutiques.

LIST of PUBLICATIONS

The main body of this thesis consists of a compilation of the following articles:

1. **Longitudinal clinical and cognitive changes along Alzheimer's disease Continuum in Down syndrome**.

   Laura Videla, Bessy Benejam, Jordi Pegueroles, María Carmona-Iragui, Concepción Padilla, Susana Fernandez, Isabel Barroeta, Miren Altuna, Silvia Valldeneu, Diana Garzón, Laia Ribas, Víctor Montal, Javier Arranz, Mateus Rozalem Aranha, Daniel Alcolea, Alexandre Bejanin, Maria Florencia Iulita, Sebastià Videla, Rafael Blesa, Alberto Lleó, Juan Fortea. Original manuscript. *JAMA Network Open*. 2022 Aug 1;5(8):e2225573 Doi: 10.1001/jamanetworkopen.2022.25573

   IF 2022: 13,366. Index SJR: 4,031. Quartile and category: Q1- Medicine (miscellaneous)

2. **Cross-sectional versus longitudinal cognitive assessments for the diagnosis of symptomatic Alzheimer's disease in adults with Down syndrome**.

   Laura Videla, Bessy Benejam, María Carmona-Iragui, Isabel Barroeta, Susana Fernandez, Javier Arranz, Sumia Elbachiri Miren Altuna, Concepción Padilla, Sílvia Valldeneu, Jordi Pegueroles, Víctor Montal, Mateus Rozalem Aranha, Alexandre Bejanin, Maria Florencia Iulita, Daniel Alcolea, Sebastià Videla, Rafael Blesa, Alberto Lleó, Juan Fortea.

   Submitted. Under review in Alzheimer´s and Dementia.

3. **Diagnostic and prognostic performance and longitudinal changes in plasma neurofilament light chain concentrations in adults with Down syndrome: a cohort study**.

   María Carmona-Iragui*, Daniel Alcolea*, Isabel Barroeta, **Laura Videla**, Laia Muñoz, Kathyrn L Van Pelt, Frederick A Schmitt, Donita D Lightner, Lisa M Koehl, Gregory Jicha, Silvia Sacco, Clotilde Mircher, Sarah E Pape, Rosalyn Hithersay, Isabel C H Clare,

Anthony J Holland, Georg Nübling, Johannes Levin, Shahid H Zaman, Andre Strydom, Anne-Sophie Rebillat, Elizabeth Head, Rafael Blesa, Alberto Lleó, Juan Fortea. Original manuscript. *Lancet Neurol.* 2021 Aug;20(8):605-614 Doi:10.1016/S1474-4422(21)00129-0

IF 2021: 59,935. Index SJR: 11,674. Quartile and category: Q1 – Clinical Neurology

4. **VAMP-2 is a surrogate cerebrospinal fluid marker of Alzheimer-related cognitive impairment in adults with Down syndrome.**
   Alberto Lleó, María Carmona-Iragui, **Laura Videla**, Susana Fernández, Bessy Benejam, Jordi Pegueroles, Isabel Barroeta, Miren Altuna, Silvia Valldeneu, Mei-Fang Xiao, Desheng, Raúl Núñez-Llaves, Marta Querol-Vilaseca, Sònia Sirisi, Alexandre Bejanin, M. Florencia Iulita, Jordi Clarimón, Rafael Blesa, Paul Worley, Daniel Alcolea, Juan Fortea, Olivia Belbin. Original manuscrit. *Alzheimer's Research & Therapy.* 2021 Jun 28;13:119. Doi: 10.1186/s13195-021-00861-0

   IF 2021: 8,823. Index SJR: 2,315. Quartile and category: Q1 – Neurology (Clinical)

LIST of ABREVIATIONS

ABC-DS	Alzheimer's Biomarker Consortium Down Syndrome
ABCG1	ATP Binding Cassette Subfamily G Member 1
Aβ	Amyloid-β
Aβ1-40	Amyloid-β 1-40 peptide
Aβ1-42	Amyloid-β 1-42 peptide
ACTC-DS	Alzheimer's Clinical Trial Consortium - Down Syndrome
AD	Alzheimer 's disease
ADAD	Autosomal Dominant Alzheimer 's disease
APP	Amyloid Precursor Protein
APOE ε4	Allele 4 of Apolipoprotein E
BACE2	β-site Cleavage Enzyme-2
CAMCOG-DS	Cambridge Cognitive Examination for Older Adults with Down's Syndrome
CAMDEX-DS	Cambridge Examination for Mental Disorders of Older People with Down syndrome and others with Intellectual Disabilities
CLSTN1	Calsyntenin-1
COVID-19	Coronavirus Disease of 2019
CSF	Cerebrospinal Fluid
DABNI	Down Alzheimer Barcelona Neuroimaging Initiative
DIAN	Dominantly Inherited Alzheimer Network
DS	Down Syndrome
DSM-V	Diagnostic and Statistical Manual of Mental Disorders Fifth Edition
DYRK1A	Dual specificity tyrosine phosphorylation regulated kinase 1A
FCSRT	Free and Cued Selective Reminding Tests
FIR	Free Immediate Recall
ID	Intellectual Disability
INCLUDE	INvestigation of Co-occurring conditions across the Lifespan to Understand Down syndrome
IQ	Intelligence Quotient
IWG	International Working Group

K-BIT	Kaufman Brief Intelligence Test
MCI	Mild Cognitive Impairment
mCRT	Modified Cued Recall Test
LME	Linear Mixed-Effect Models
MMSE	Mini-Mental State Examination
MRI	Magnetic Resonance Imaging
NfL	Neurofilament light chain
NIA	National Institute on Aging
NIA-AA	National Institute on Aging and Alzheimer's Association
NIH	National Institute of Health
NLGN2	Neuroligin-2
NPI	Neuropsychiatric Inventory
NPTX2	Neuronal Pentaxin 2
NRXN2A	Neurexin-2A
NRXN3A	Neurexin-3A
PET	Positron Emission Tomography
PSEN1	Presenilin1
PSEN2	Presenilin2
p-Tau	Phosphorylated Tau
REST	RE-1-silencing Transcription Factor
ROC	Receiver-Operating Characteristic
SD	Standard Deviation
SE	Standard Error
SIMOA	Single Molecule Array
SOD-1	Superoxide Dismutase 1
SPIN	Sant Pau Initiative on Neurodegeneration
STX1B	Syntaxin-1B
Thy-1	Thymus cell antigen 1
TIR	Total Immediate Recall
T21RS	Trisomy 21 Research Society
T-tau	Total Tau
VAMP-2	Vesicle-Associated Membrane Protein 2

INTRODUCTION

Down syndrome (DS) or trisomy 21 is the most common cause of intellectual disability (ID) of genetic origin. The triplication of the genes coded in chromosome 21, most importantly the triplication of the amyloid precursor protein (APP), lead to the universal presence of Alzheimer's disease (AD) pathological hallmarks in adults with DS by the age of 40, and, subsequently, to an ultra-high risk to develop AD dementia. Therefore, AD is now the main medical problem and cause of death in this population.

The clinical diagnosis of dementia in individuals with DS is a challenge due to the associated neurodevelopmental ID, and the lack of validated clinical criteria and neuropsychological normative data to diagnose symptomatic AD. Most importantly, there are no treatments to prevent or stop AD in this population, and adults with DS have historically been excluded from AD clinical trials denying them the opportunity to benefit from potential treatments.

However, the landscape of AD research in DS, and our understanding of the disease, has dramatically changed during the past 10 years. This thesis, which was begun in 2017, has contributed to this change and challenged some commonly assumptions held at that time: (i) A significant proportion of adults with DS will not develop the clinical symptoms of AD despite the universal and early neuropathological changes. (ii) The clinical course of AD and its onset is very variable and different from that of sporadic AD. Indeed, the prevailing view of the clinical presentation was that of an atypical form of AD characterized by changes in behavior, executive functions, and activities of daily living. (iii) Most important for this thesis, the diagnosis of symptomatic AD did not include neuropsychological criteria, as cognitive performance was considered too variable and there were no normative values to diagnose symptomatic AD, the clinical diagnostic gold standard for symptomatic AD was (and is) the clinical criteria of experienced physicians.[1] (iv) Finally, there was very limited experience regarding the utility of AD biomarkers in this population.

1. Down syndrome

1.1 Epidemiology of Down syndrome

Down syndrome (DS) affects about 6 million people worldwide,[2] 417,000 in Europe.[3] Mother´s age is the main risk factor for having a baby with DS, especially above 35 years. The increase of mean maternal age has surpassed that of the elective pregnancy termination rate, resulting in an increase in the incidence of DS in the last decades from 16 of 10.000 births to 23 of 10.000 births in 2015.[4] On the other hand, improvements in health care and management of co-occurring illnesses have greatly increased the life expectancy of in this population, from 25 years in the 1983 to 60 years in the 2020s.[5] These trends have dramatically changed the epidemiology of the population with DS. Contrary to a commonly held view, there are more people living with DS than 20 or 40 years ago, and it is a fast aging population, with more people in their fourth, fifth, and sixth decades of life (Figure 1). Hence, DS is no longer a childhood disability[6] and there is an increased need to focus on aging challenges in this population, most importantly AD.

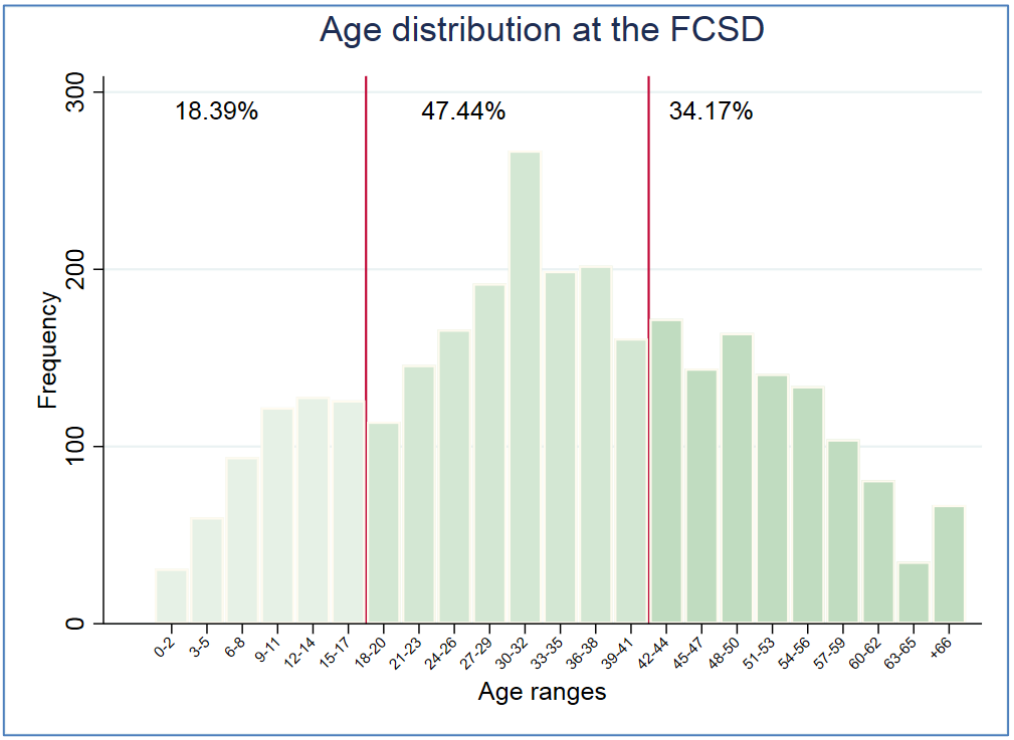

Figure 1. Age distribution of the population with DS attended at the *Fundació Catalana Síndrome de Down* (FCSD) showing more than 80% of adults, of whom 35% are over 41 years old.

1.2 Etiology of Down syndrome

DS is the most common cause of Intellectual Disability (ID) of genetic origin and is caused by the triplication of chromosome 21.[7] Most cases of DS (~95%) are caused by a parental meiotic nondisjunction in the germinal cells, which results in a complete trisomy.[8] Consequently, the embryo has three copies of chromosome 21 in each cell of its body. Much less frequently, in approximately 3 to 4% of cases, DS is caused by a chromosomal translocation. This occurs when an extra copy of chromosome 21 (full or partial) attaches to another chromosome (usually chromosome 14, 21, or 22). In 1 to 2% of cases, DS is caused by mosaicism due to an error in mitosis, in which individuals have some cells containing the usual 46 chromosomes and others containing 47 with an extra chromosome 21. Finally, in less than 1% of cases, DS is caused by a partial trisomy of a delimited segment of chromosome 21. The clinical features of nondisjunction and translocation do not differ significantly, but mosaicism and partial trisomy 21 are usually associated with milder neurodevelopmental disturbances (and risk for co-occurring conditions).[7,9]

Two main hypotheses had been proposed to explain the biological perturbations that underlie phenotypic manifestations of DS. First, the gene-dosage effect hypothesis focuses on the direct effects of overexpressed chromosome 21 genes and their downstream consequences. Second, the developmental instability hypothesis posits that global disturbance of gene expression arising from the extra chromosome 21 results in disruption of overall biological homeostasis beyond chromosome 21. More recently, a genome imbalance hypothesis has been proposed, combining these two previous hypotheses.[8]

The increased gene expression in individuals with trisomy 21 leads to neurodevelopmental problems, early aging and an ultra-high risk to develop AD dementia. The dual specificity tyrosine phosphorylation regulated kinase 1A (DYRK1A) is one of the most studied genes in chromosome 21. It codes to a pleiotropic kinase, which has an important role in neurogenesis and synaptogenesis, as well as tau phosphorylation. DYRK1A is especially related to some of the characteristics of DS, such as ID and motor skills problems. Likewise, DYRK1A is related to the underexpression of the gene RE-1-silencing transcription factor (REST), which modulates the expression of other important genes for correct neurological development. REST underexpression is related to a reduced number of neurons, and aberrant neural connections.[10–12] Most importantly, the Amyloid Precursor Proteine (APP) gene, which encodes the amyloid precursor protein, is related with Alzheimer's disease (AD) pathology. Amyloid- β (Aβ) plaques, one of the main pathological hallmarks of AD, originate from proteolytic cleavage

of APP.[13] β-site cleavage enzyme-2 (BACE2), also overexpressed in DS, might also contribute to APP protein cleavage, and thus to the amyloidogenic pathway. The ATP Binding Cassette Subfamily G Member 1 (ABCG1) gene, which is a cholesterol transporter, affects the proteolytic processing and subcellular distribution of APP, increasing production of Aβ peptide.[14] Finally, as a response to oxidative stress, the enzyme superoxide dismutase 1 (SOD-1) is also overexpressed and it seems to also play an important role in early aging in DS.[15]

The genetic imbalance caused by chromosome 21 triplication leads to alterations in neurogenesis and synaptogenesis that have important consequences on brain development, causing intellectual disability as the most common feature in DS.[16] The intensity and manifestations of ID are intrinsically individual and largely unpredictable. However, there are some general common features along most individuals with DS, including brain structures, as well as medical, physical and cognitive phenotypes.

1.3 Neuroanatomy of Down syndrome

Brains of people with DS have a reduced number of neurons and reduced neuron sizes. However, despite these common features, the neurobiology of this population is not static, and changes with age throughout the whole lifespan.[17]

Post-mortem observations and in vivo neuroimaging studies indicate that people with DS have smaller overall brain volumes, brachycephaly, ventriculomegaly, a simplified appearance of the sulci, and a narrow superior temporal gyrus.[18,19] This reduction in overall brain size is not only due to a generalized reduction, but most prominent in specific brain regions, with smaller volumes in frontal and temporal areas, cerebellum, and basal forebrain when compared with euploid individuals. By contrast, subcortical areas, such as the lenticular nuclei and the posterior parietal and occipital cortical grey matter, have relatively normal brain volumes.[20]

A prominently reduced volume of the cerebellum from early life is universal in individuals with DS. A smaller cerebellum may be responsible for the hypotonia, the delay in motor coordination, gait disturbances, and some speech difficulties, such as speech articulation. The frontal atrophy, especially the prefrontal cortex, is also common and may explain failures in working memory, executive dysfunction, attention deficits, low performance in switching tasks and a greater tendency to perseveration. Smaller Hippocampi are also a salient feature of the brain in individuals with DS and is related with deficits in long-term memory. These neurodevelopmental abnormalities persist along the whole lifespan.[21–24] Less consistent volume reductions have been found in the parietal and temporal lobes. This may explain the

variability observed in the development of language skills in people with DS. Nonetheless, language is affected in all individuals with DS. Amygdala volume in individuals with DS does not differ significantly from those of controls after adjustment for total brain volume.[18]

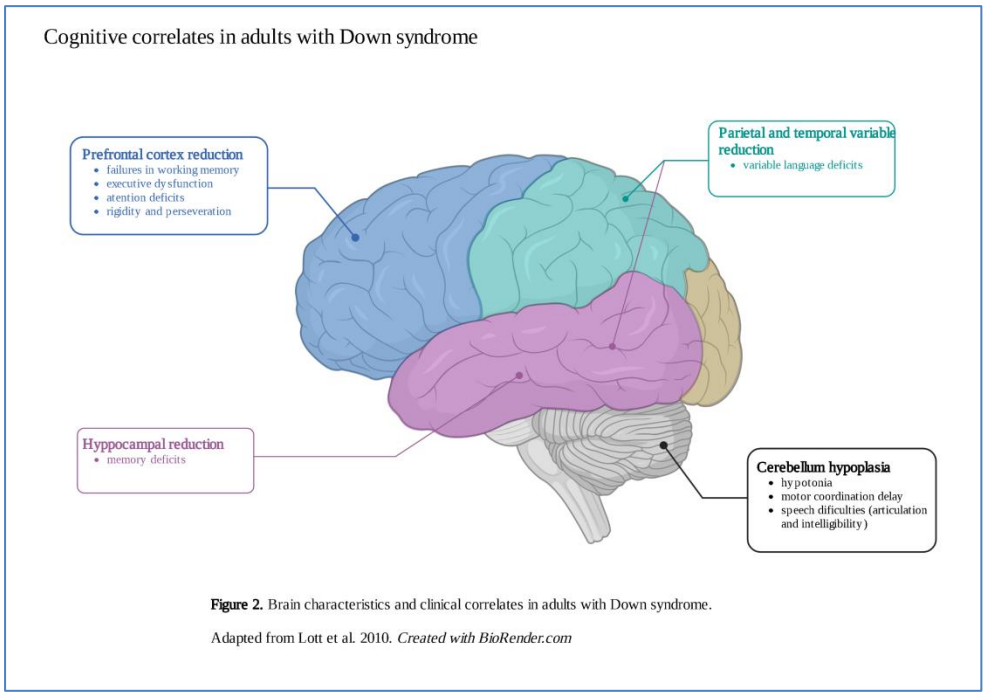

Figure 2. Brain characteristics and clinical correlates in adults with Down syndrome.

Adapted from Lott et al. 2010. *Created with BioRender.com*

1.4 Intellectual disability in Down syndrome

The most salient feature of DS is the intellectual disability (ID). The Diagnostic and Statistical Manual of Mental Disorders Fifth Edition (DSM-V) defines ID as a neurodevelopmental disorder that begins in childhood and is characterized by intellectual difficulties as well as difficulties in conceptual, social, and practical domains. The current diagnostic criteria include:

> "A) Deficits in intellectual functioning, such as reasoning, problem-solving, planning, abstract thinking, judgment, academic learning, and learning from experience, confirmed by both clinical assessment and individualized standardized intelligence testing.
>
> B) Deficits in adaptive functioning that result in a failure to meet developmental and sociocultural responsibility. Without ongoing support, the adaptive deficits limit functioning in one or more activities of daily life, such as communication, social participation, and independent living, across multiple environments, such as home, school, work, and community.

C) Onset of intellectual and adaptive deficits during the developmental period."[25]

DSM-V requires classification of the intellectual disability severity into "mild," "moderate," "severe" or "profound" categories, focusing on daily skills and not only on a specific Intelligence Quotient (IQ) as they did in previous versions of DSM:

- "Mild ID: individuals with mild ID are slower in all areas of conceptual development and social and daily living skills. These individuals can learn practical life skills, which allow them to function in ordinary life with minimal levels of support.
- Moderate intellectual disability: can take care of themselves, travel to familiar places in their community, and learn basic skills related to safety and health. Their self-care requires moderate support.
- Severe intellectual disability: manifests as major delays in development, and individuals often have the ability to understand speech but otherwise have limited communication skills (Sattler, 2002). Despite being able to learn simple daily routines and to engage in simple self-care, individuals with severe ID need supervision in social settings and often need family care to live in a supervised setting such as a group home.
- Profound intellectual disability: often have congenital syndromes (Sattler, 2002). These individuals cannot live independently, and they require close supervision and help with self-care activities. They have very limited ability to communicate and often have physical limitations. Individuals with mild to moderate disability are less likely to have associated medical conditions than those with severe or profound ID."[25]

ID can be estimated and quantified through intelligence tests to estimate an IQ. Despite the limitations of IQ, as for example the antiquate scientific rationale of most intelligence batteries,[26] IQ is currently a common way to represent intellectual functioning when measured using appropriate, standardized, and individually administered assessment instruments. The mean IQ test score in the general population is 100 with a standard deviation (SD) of 15 [SD 85-115]. Scores conform to a normal distribution curve. That means that nearly 70% of the population scores within plus or minus 15 points of the average score. Scores over 140 are

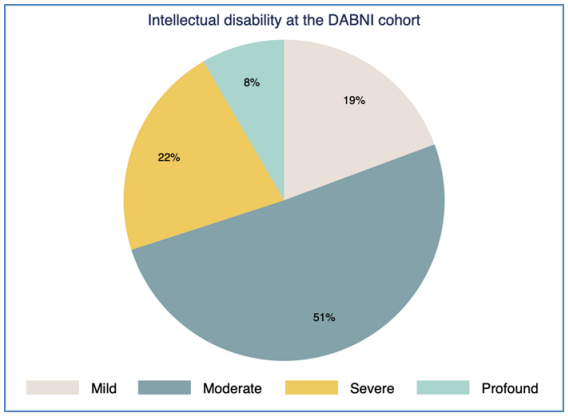

Figure 3: Prevalence of the level of intellectual disability in the DABNI cohort of adults with Down syndrome

considered high intellectual functioning and IQ scores below 70 are considered low intellectual functioning, and may suggest ID. Approximate equivalences between IQ and ID have been proposed; IQ scores between 50/55 and 70 corresponds to mild ID, the range between 35 to 49 to moderate ID, and scores below 35 to severe and profound ID. The most common forms of ID in DS are mild and moderate (Figure 3)

Infants and children with DS reach developmental milestones in the same linear pathway as their non-trisomic peers, but with a delay and significant variation depending on the milestone. Thus, they are not greatly delayed in smiling and social interaction, but motor development and language learning are further delayed.[27] As mentioned, babies with DS follow the same steps of motor development as other babies but need more time to develop strength and motor control as well as more practice to correctly control these skills. Initially, all motor skills are less precise, but improve with practice. For example, they require more time to develop balance, to stand, and to walk.[28] Children with DS have a good non-verbal communication by means of gestures, but show greater difficulty with speech, so they typically understand more than they can say. Finally, in general, they are better at processing and remembering visual than verbal information.[27]

Cognitive development in individuals with DS spans throughout childhood, adolescence, and early adulthood,[29,30] and is followed by a gradual loss of abilities[29,31] later in life commonly associated with Alzheimer disease (AD).[32] Individuals with DS demonstrate a consistent deficit in the processing of verbal information relative to visual information. Cognitive functioning evolves during life and is impacted by several comorbid factors, such as sensory impairments, seizures, sleep disruption, and other medical and psychiatric conditions.[33] However, we summarize the general cognitive phenotype that has been described in DS:

Language: Language is a complex cognitive domain that can be subdivided into language comprehension and expression, and, depending on the sensory input pathway, can be oral or written. In general terms, comprehension involves different processes than expression. Studies have demonstrated that nonverbal skills are less compromised than verbal skills throughout development of individuals with DS.[34]

The development of verbal abilities in children with DS decelerates in adolescence and expressive language is delayed relative to comprehension.[29,35] Early language milestones (e.g., babbling) are typically met within an age-expected range.[36] However, infants show reduced vocal reactivity and responsiveness to the environment, generally resulting in a delayed acquisition of the child's first words[37] and persistent delays in language skills once the child

reaches age five.[38] Language delays are more pronounced at school age and maintained along the lifespan, with prominent difficulties in expressive syntax and phonological processing.[37] Regarding receptive vocabulary, simple comprehension is quite preserved during adolescence; however, the ability to comprehend more complex language syntax may plateau in late childhood or early adolescence.[39]

In adulthood, language deficits in articulation, phonological processing, and morphosyntax remain diminished, and semantic, pragmatic, and communicative intent are relatively preserved.[37] Speech comprehension and production slow further with age, language becomes less fluent, word discrimination becomes more difficult, and speech organization/word retrieval problems emerge. Some of these difficulties may be attributed in part to age-related changes in hearing, auditory discrimination, and less efficient respiratory support for speech.[40] All these language deficits contribute to dysfunction in other cognitive domains.

Memory: Memory is defined as a neurocognitive function that allows encoding, consolidating, retaining, storing, retrieving, and recalling previously stored information.[41] Individuals with DS, both children and adults, can learn and acquire new skills throughout their life; however, the rate of learning and the range of skills acquired often differ from children with neurotypical development in both short-term and long-term memory.[42–44] Non-verbal and observational learning and memory are strengths when compared to verbal skills. They also have deficits in verbal and non-verbal long-term memory at all ages[37] and at several levels including encoding, retrieval,[45] and consolidation.[20]

Executive Functions: This cognitive domain includes a wide range of cognitive functions with different levels of complexity. The lower levels involve the regulatory components of behavior and cognition, including aspects of attention, inhibition, and processing speed, while the higher levels include information processing as strategic planning, impulse control, organized search, flexibility, self-monitor behavior, and future planning and organization.[46]

Overall, individuals with DS show executive function impairments that extend beyond those observed in individuals with other ID of unknown etiology with a comparable IQ.[37] Both children and adults with DS show poorer sustained attention and a deficit in inhibitory control,[47] a slow speed of reaction time and information processing, and show significantly more difficulty with shifting, planning, and working memory. They also show perseverative deficits, and these deficits increase when using verbal stimulus.[37]

Visuospatial abilities: Visuospatial abilities, such as visual processing, visuospatial short-term memory, and visuo-construction, are relatively preserved in individuals with DS compared to verbal skills, and they remain preserved until old ages.[48]

In brief, the main cognitive trait in adults with DS is ID, which ranges from mild to profound, being mild and moderate ID the most prevalent. The most affected cognitive domains in young adults with DS include language (especially verbal expressive language), memory, executive function, and motor coordination. Certainly, these alterations can vary from one individual to another, both in quality and intensity, as well as in their evolution with age.[16]

1.5 Physical phenotype and medical co-occurring conditions in Down syndrome

The genetic imbalance in people with trisomy 21 also leads to some common physical and medical conditions. The most affected body systems are the musculoskeletal, neurological, and cardiovascular systems. It is noteworthy that, the level of Intellectual Disability (ID) does not correlate with other characteristics such as the intensity of facial features or the severity of medical conditions.[7]

Individuals with DS often have a short stature, a characteristic facial appearance that includes a flattened appearance to the face, up slanting palpebral fissures, small ears, a short neck, and macroglossia, they usually also have small hands and feet and a single crease across the palms of the hands, and another typical trait is hypotonia.[7] People with DS have an increased risk of developing some medical co-occurring conditions including congenital heart diseases, airway and pulmonary disorders, growth disorders, overweight and obesity, hematologic and oncologic disorders, autoimmune disorders, musculoskeletal disorders, sleep disorders, thyroid abnormalities, celiac disease, gastrointestinal anomalies, sensory deficits and epilepsy.[49] Importantly, due to the triplication of the APP gene and the increase in life expectancy during recent decades, AD is emerging as a major health problem in adults with DS.

2. Alzheimer's disease

AD is a prolonged and progressive disease that begins with pathophysiological changes in the brains of affected individuals up to 20 years before any clinical manifestations are observed.[50] The main neuropathological hallmarks of AD include the aggregation of soluble fragments of Aβ, mostly Aβ1-42 peptides, and the accumulation of hyperphosphorylated tau proteins forming extracellular amyloid plaques and intracellular neurofibrillary tangles respectively.

These two proteinopathies lead to progressive neurodegeneration, including neural and synaptic loss.[51] Synapse loss is an early event in AD[52] that precedes neural death and clinical symptomatology. However, it has also been proposed as one of the primary pathological correlates of cognitive dysfunction.[53] Alongside these main hallmarks, gliosis, neuroinflammation, and vascular dysfunction are also present, which reflects the complexity of AD.[54]

2.1 Sporadic, autosomal dominant Alzheimer's disease and Alzheimer's disease in Down syndrome

The most common form of AD in the general population is sporadic AD, which accounts for 99% of cases. Symptoms of sporadic AD are commonly detected after the seventh decade. Although its main etiology is still unknown, it seems to be caused by a complex combination of genetic factors, environmental conditions, and lifestyle. AD is a complex disease that results from the interaction of genetic and environmental risk factors. The strongest risk factor is age, both the incidence and prevalence increase exponentially with age.[55] The most important genetic risk factor for sporadic AD is the allele 4 of apolipoprotein E (APOE ε4),[56] but several of other genetic risk factors have been identified in large genome wide association studies (GWAS) studies. These other genes confer only small increases in the risk to develop AD, but are providing unvaluable information about the pathophysiology of the disease. There are other modifiable risk factors that account to up to 40% of the risk, including less education, hypertension, hearing impairment, smoking, obesity, depression, physical inactivity, diabetes, low social contact, excessive alcohol consumption, traumatic brain injury, and air pollution.[57] Targeting these risk factors is essential as many dementia cases would be prevented.[58]

On the other hand, autosomal dominant Alzheimer's disease (ADAD) accounts for less than 1% of all AD cases. Unlike sporadic AD, ADAD forms have an early onset presentation, before the age of 65, and have, so far, been linked to mutations in three genes, presenilin1 (PSEN1), presenilin2 (PSEN2), and APP.[59]

There are genetic and biological differences between ADAD and sporadic AD. Sporadic AD has been associated with reduced Aβ42 clearance and ADAD with increased Aβ42 production; however, the biochemical consequences are similar, with accumulation of Aβ in the brain playing an early role.[60] More importantly, they have similar neuropathological and clinical features.

As mentioned, people with DS have an extra copy of chromosome 21, which encodes the APP gene. Because of this triplicated gene, individuals with DS overproduce Aβ peptides with consequent accumulation in their brains along their lifespan. Moreover, it is not clear if there is also a failure in Aβ clearance pathways.[61] In the context of DS, the triplication of APP is sufficient and necessary to produce AD. It is sufficient because there are rare families with a triplication of the APP gene (and no DS) and they develop early onset AD.[62] It is necessary because there are rare cases of DS in which the region of APP is not included in the triplication and do not develop AD pathology or symptomatology.[63] Consequently, in 2014, DS was considered for the first time as an atypical genetic form of AD.[64] In agreement with this conceptualization, amyloid plaques and tau neurofibrillary tangles are present in all individuals with DS by the age of 40 years.[65] This, together with the increasing life expectancy in this population, leads to an exponentially increased lifetime risk of developing dementia.[66] There is very limited information regarding genetic and disease-modifying risk factors associated with AD in DS. In this sense, one study found that ApoE4 haplotype accelerated amyloid deposition and mortality in DS.[67] In this line, some studies found that known risk factors for sporadic AD as APOEε4, low education, hypertension, diabetes, dyslipidemia, and obesity, were associated with slower cognitive decline in ADAD.[68]

2.2 Alzheimer's disease diagnostic criteria

AD is a progressive and insidious disease. Its main clinical manifestation is dementia, which is defined as a loss of cognitive functioning (thinking, remembering, and reasoning) to such an extent that interferes with a person's daily living activities and life routine. Different disorders and factors can contribute to developing dementias such as Parkinson's disease, vascular conditions, or frontal lobe degeneration. However, AD is the most prevalent cause of dementia worldwide in older individuals from the general population (aged over 65 years)[69] accounting for 50-60% of people with dementia. Official death certificates recorded 121.499 deaths from AD in 2019 in the US. It was officially listed as the sixth-leading cause of death in the United States that same year, and the seventh-leading cause of death in 2020 and 2021 when COVID-19 entered the ranks of the top ten causes of death.[70]

In 1984, the diagnosis of AD was based on the National Institute of Neurological and Communicative Diseases and Stroke/Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria.[71] These criteria described a typical clinical picture, recommended laboratory testing to exclude other causes of dementia, and required post-mortem neuropathological examination for a definitive diagnosis of AD.[72] The advances in the field of

biomarkers led to the redefinition of AD. Currently, there are no universally accepted diagnostic criteria, and research criteria for AD have changed since the beginning of this thesis. The most used are the revised International Working Group (IWG) criteria,[64] and the National Institute on Aging and Alzheimer's Association (NIA-AA) AT(N) classification.[73] Despite some differences, both classifications include pathophysiological biomarkers for AD.

The IWG criteria aimed to improve the diagnostic framework, and required the presence of an appropriate clinical AD phenotype (typical or atypical) and a pathophysiological biomarker consistent with the presence of Alzheimer's pathology. Importantly, for the first time these criteria included DS as an atypical form of AD, with early occurrence of a distinct dementia characterized by behavioral changes and executive dysfunction as the initial symptoms.[64] In 2018, the NIAA reviewed the diagnostic recommendations for AD to shift from a clinicobiological definition to a biological framework in a research context.[74] They grouped biomarkers into those of β-amyloid deposition, pathologic tau, and neurodegeneration, creating the AT(N) classification widely used in the current research framework. This AT(N) classification system groups different biomarkers (imaging and biofluids) by the pathologic process of each measure. AD biomarkers will be explained further in the following section.

Although these classifications have some limitations, the evolution in the conceptualization of AD with the incorporation of biomarkers resulted in the definition of "preclinical AD". This term defines those individuals with positive AD biomarkers indicating the presence of Aβ and tau pathology, but with no clinical symptoms of AD. Thus, currently AD is conceptualized as a continuum with 3 main stages: preclinical, prodromal, and dementia.[74] Individuals progress from the preclinical stage, characterized by normal cognition and abnormal brain biomarkers, to mild cognitive impairment or prodromal AD, until activities of daily living are impaired, which characterize the AD dementia stage.

Despite the ultra-high risk of AD dementia in people with DS, there is still a lack of validated diagnostic criteria. Clinical diagnosis of symptomatic AD in this population is problematic due to the coexistent ID, which is variable between individuals. The major challenge is detecting and distinguishing AD-related cognitive decline from the associated neurodevelopmental ID. Moreover, classic cognitive tests commonly used in the general population, such as the Folstein Mini-Mental State Examination (MMSE)[75] or the Free and Cued Selective Reminding Tests (FCSRT),[76] are not useful to assess people with ID, as most individuals with DS will score at floor levels. On the other hand, most adults with DS and symptomatic AD will not express concerns about their cognitive problems, and their families, caregivers, and clinicians are

usually unaware. Consultations for cognitive decline are often only done when activities of daily living are substantially affected, or when behavioral problems emerge, which usually happen in more advanced stages of the disease. In some instances, there is absence of a reliable caregiver who can accurately and comprehensively inform medical professionals of the patient's changes, further complicating the evaluation and diagnosis.[77]

To address these issues, the National Task Group on ID, comprising specialists in the evaluation of adults with ID, proposed some specific dementia guidelines in 2013. They recommended a stepwise and comprehensive assessment of suspected cognitive decline. Given the individual differences in premorbid ID levels, the National Task Group emphasized clinical history as a key point of the assessment. It is important to collect robust information on individuals' premorbid functioning, as well as within-person change over time to detect AD-related cognitive decline. Another important focus is reviewing medication and to assess potential psychosocial problems.[78] They also recommended a physical exam and cognitive assessment whenever possible. However, and key to this doctoral thesis, due to the heterogeneity of ID, they stressed the ineffectiveness of population norms in percentiles, standards, or generalized sets of expectations when assessing an individual for dementia. They proposed that the assessment of cognitive decline should always be individualized and patient-specific, with judgments based on deterioration from the patient's own individual baseline level of ID, function, and achievement. Finally, the National Task Group urged health care professionals to arrive at the diagnosis of dementia systematically and thoughtfully, so as not to prematurely close a window of opportunity to discover potentially modifiable and treatable coexisting conditions.[78]

2.3 Alzheimer's disease-related cognitive decline

The typical cognitive expression of Alzheimer's disease is closely related to the topography of the neuropathological changes in the brain, mainly those of tau.[79] The earliest neurofibrillary changes usually occur in medial temporal lobe structures (e.g., hippocampus, entorhinal cortex). These initial changes are followed by the spreading of atrophy in the brain cortex through the bilateral parietal and frontal lobes.[80]

In accordance with the topography of AD neuropathology, AD-related cognitive decline in the general population typically starts with memory problems, with profound deficits in the encoding and storage of new information (episodic memory). This memory deficit can be optimally detected with memory tests that enhance mnemonic retrieval through encoding specificity techniques such as the FCSRT.[76] After the memory impairment starts, other

cognitive domains are progressively affected, particularly executive function, attention, and praxis.[79] The cognitive profile described in ADAD forms does not differ substantially from that described in sporadic AD.[81]

In addition to the typical amnestic presentation, we now know that there are other cognitive presentations, known as “atypical AD”, in which the most prominent symptom might be language impairment (word-finding), visuospatial deficits (agnosia), or executive dysfunction.[79]

Despite a similar topographical distribution of AD neuropathology in sporadic AD and DS, clearly described in the 80s, the characterization of the cognitive presentation of AD in DS at the beginning of this PhD was controversial, as different presentations had been described (amnestic, executive dysfunction, behavioral),[82,83] particularly in the early stages. Earlier studies had suggested a preferential non-amnestic presentation akin to atypical AD in sporadic AD with prominent behavioral and psychological symptoms of dementia and impairment of executive functions preceding memory decline.[64,82] In fact, in the IWG criteria of 2014, DS was considered to have this atypical presentation of AD. This is not surprising in a context where the individual with DS does not complain about his or her cognitive problems and the caregivers consult professionals only when the cognitive decline interferes in daily living activities or when behavioral and psychological symptoms are present and prominent. All these factors might overshadow the initial typical clinical presentation of AD, typically arising as episodic memory impairment in the general population.

An additional factor contributing to the complexity of symptomatic AD diagnosis is the fact that AD clinical symptoms can be obscured by coexisting medical conditions that might affect cognition. As we mentioned before, it is of paramount importance to explore possible contributing factors that are potentially correctable before establishing a dementia diagnosis. These possible co-occurring conditions must be routinely and actively explored in individuals with DS to rule out sensory deficits (hearing and visual loss), metabolic disturbances (vitamin B12 deficiency, thyroid dysfunction, etc.), coexisting mood disorders or adaptive disorders, pharmacologic interferences, obstructive sleep apnea, seizures, or psychosocial and environmental stressors.[78]

In this sense, advances in the field of biomarkers for AD in the general population can be especially useful in this high-risk population of individuals with DS as a diagnostic and predictive tool for AD.

3. Alzheimer's disease biomarkers

A biomarker is a biological parameter that can be objectively measured and evaluated as an indicator of normal or pathological states or pharmacological responses to therapeutic intervention.[84] Until 2007 (first IWG criteria) and 2011 (NIA-AA criteria), AD diagnosis was based on clinical symptoms and confirmation was only possible through post-mortem neuropathological confirmation. The IWG and the NIA-AA criteria incorporated biomarkers for research purposes. However, the use of AD biomarkers in clinical practice is widespread in specialized memory clinics today.[85] AD biomarkers have proven to be useful not only to diagnose AD, but also to identify subjects at the preclinical stage of AD, to prognosticate patients or to track the impact of therapeutic drugs. Post-mortem studies indicate that adults with DS have a similar pattern of cerebral atrophy, as seen in the early stages of sporadic AD.[24,86] Nevertheless, only a few studies with very small sample sizes studying in vivo biomarkers had been performed by 2017, at the beginning of this PhD. One of these, with a sample size of 12 non-demented adults with DS, found similar plasma and neuroimaging biomarker changes in DS and sporadic AD.[87]

As shown in Figure 4, AD biomarkers can be grouped in several ways depending on the modality or technique used for its collection (biofluids vs. imaging); according to the information they are providing (amyloid vs. tau); and state or stage biomarkers (state biomarkers are those reflecting AD pathophysiology, and stage biomarkers are those underlying clinical worsening or AD progression). The most investigated biomarkers in AD can be grouped in:

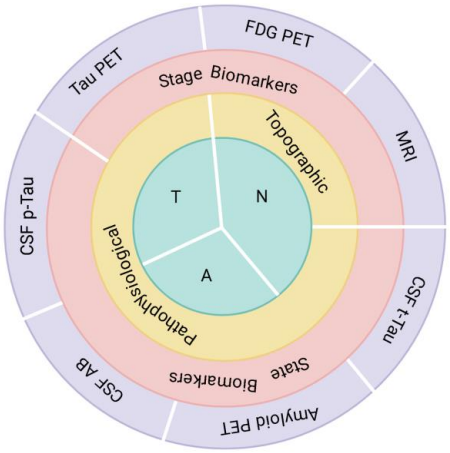

Figure 4. AD-related biomarkers grouped by different classification systems. From inside to outside: AT(N) (Jack et al. 2016), State vs. Stage (Blennow et al. 2003; Dubois et al 2016), Pathopysiological vs. Topographic (Dubois et al. 2014)

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3.1 Biochemical biomarkers

The 2 biological fluids most widely studied in AD biomarkers are peripheral blood and cerebrospinal fluid (CSF).

CSF is a fluid surrounding the brain that can provide important biochemical information regarding the brain status[88] and can safely be obtained through a lumbar puncture.[89] The biochemical signature of AD in CSF is composed of reduced levels of Aβ42, due to the entrapment of Aβ in amyloid plaques; and elevated levels of total tau (T-tau) and phosphorylated Tau (p-Tau) reflecting cortical tangle formation and cortical neuronal loss.[90] There are many studies assessing the diagnostic accuracy of these biomarkers in sporadic AD[91] and ADAD, [cita] but very few in DS. Lumbar punctures are rarely performed in individuals with DS. Until 2017, very few studies had evaluated core CSF AD biomarkers (CSF Aβ42, T-tau, and p-tau) in this population.[92–95] Despite methodological limitations, these studies suggested that CSF Aβ1–42 negatively correlated with age in DS, and that it was lower than in healthy controls from the general population. Tau showed a positive correlation with age but did not differ between DS and non-trisomic controls.

In parallel to the development of this thesis, a new biomarker for neurodegeneration gained enormous attention, neurofilament light chain (NfL) levels. NfL is a scaffold protein of neuron cytoskeleton that, in the context of neurodegeneration, is released into the CSF and blood and it can capture neuronal damage in a wide variety of neurologic conditions.[96] The exact mechanism by which NfL is released from damaged neurons is not completely understood but elevated levels of NfL are present in neurodegenerative disorders, including AD.[97] At the beginning of this thesis, no studies of NfL in DS had been performed.

Although the core CSF AD biomarkers reflect central pathogenic mechanisms of the disease, novel biomarkers to monitor additional important pathological mechanisms in AD are emerging. One important component of AD pathological change and pathophysiology is synaptic dysfunction and degeneration.[88] Synaptic proteins can be detected in CSF. Current research suggests that CSF synaptic proteins are altered early in AD, reflecting the response of synapses and neurons to Aβ-mediated damage. During the development of this PhD, our group described and patented a set of 9 synaptic proteins that are specifically expressed at the synapse, and show alterations that precede clinical symptoms and markers of neurodegeneration in sporadic AD: Neuronal Pentaxin 2 (NPTX2), Calsyntenin-1 (CLSTN1), Neuroligin-2 (NLGN2), Neurexin-2A (NRXN2A), Neurexin-3A (NRXN3A), Syntaxin-1B (STX1B),

Thymus cell antigen 1 (Thy-1) and vesicle-associated membrane protein 2 (VAMP-2).[98] However, the field of synaptic biomarkers was unexplored in DS.

In 2017, when this thesis started, the utility of blood-based biomarkers for AD was questionable and no studies had validated their clinical utility.[99–101] However, in 2018, Nakamura et al. revolutionized the field by demonstrating the potential clinical utility of plasma biomarkers in predicting brain amyloid-β burden at an individual level by measuring plasma amyloid-β through immunoprecipitation coupled with mass spectrometry.[102] Technical advances enabled the accurate measurement of P-Tau levels, which proved suitable biomarkers for AD diagnosis and screening in the general population.[103] Finally, ultrasensitive platforms made possible the measurement of plasma NfL concentrations that were shown to correlate with cognitive impairment in the general population.[97] A study exploring the diagnostic performance of plasma biomarkers for AD in DS showed promising results for increased levels of Aβ40 and Aβ42 concentrations when compared with non-demented individuals with DS,[104] but no studies had explored the potential validity of NfL in DS.

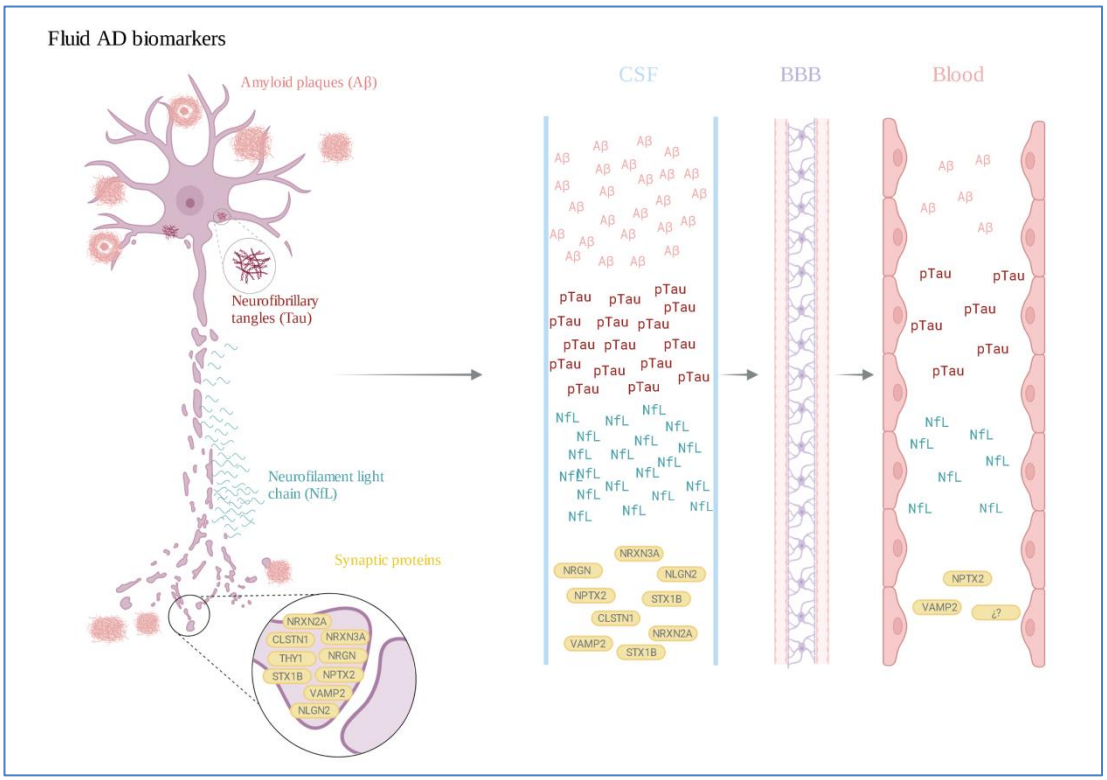

Figure 5: Biofluid-based biomarkers in Alzheimer's disease. Alzheimer's disease has a complex pathophysiology, and some biomarkers can be measured in CSF and blood (Aβ, pTau and NfL). Presynaptic proteins can be measured in CSF, but further studies are needed to explore their validity in blood.
CSF= Cerebrospinal fluid; BBB= Blood Brain Barrier; Aβ=amyloid β; pTau=phosphorylated tau; NfL=neurofilament light chain; NPTX2= Neuronal Pentaxin 2; CLSTN1=Calsyntenin-1; NLGN2=Neuroligin-2; NRXN2A=Neurexin-2A; NRXN3A=Neurexin-3A; STX1B=Syntaxin-1B; Thy-1= Thymus cell antigen 1; VAMP-2= vesicle-associated membrane protein 2.

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3.2 Neuroimaging biomarkers for AD

In vivo neuroimaging in humans can provide a better understanding of some diseases, such as AD. The most used neuroimaging techniques in the field of AD are Magnetic Resonance Imaging (MRI) and Positron Emission Tomography (PET), but since these biomarkers are not the focus of this thesis, they will be explained in limited detail.

MRI is a high imaging resolution, non-invasive technique that produces images of brain structure. The typical AD signature observed with MRI is atrophy in hippocampal volume[64] and entorhinal cortex in both sporadic AD and ADAD.[105] The atrophy pattern associated with AD in DS also involves posterior dominant cortical thinning in a similar pattern to what is seen in sporadic AD and ADAD.[20]

On the other hand, a PET scan is a nuclear medicine technique that measures the metabolic activity of cells. In the context of AD, different tracers have been developed to detect different pathological hallmarks of the disease. Similar patterns of cerebral Aβ deposition and hypometabolism have been widely described in sporadic AD and ADAD.[106] Of note, at the beginning of this thesis, in DS the most robust AD-pathophysiological studies were conducted with amyloid-PET.[107]

In summary, neuropsychological testing in the field of ID was traditionally limited to the quantification of the global developmental delay through IQ tests.[108] Only five years ago, although diagnostic criteria for dementia in adults with ID focused on recognizing cognitive, functional, or behavioral changes concerning the individual-premorbid level of functioning, formal neuropsychological testing was most often incorporated in the diagnostic process. The most salient limitations were the lack of thresholds or normative data to guide a neuropsychological diagnosis of symptomatic AD in DS despite the development of some adapted neuropsychological tools over the past decades and the emphasis on intra-individual objective longitudinal decline. Moreover, the advances in the field of AD biomarkers had hardly been investigated in very small studies.

This thesis tried to fill these gaps focusing on the diagnostic performance of two commonly used neuropsychological instruments and plasma and CSF biomarkers with the aim to advance and more robustly diagnose symptomatic AD in the DS population. There is a growing need for a better understanding of the pathological process of AD in DS and to find clinical biomarkers to enable accurate and early diagnosis in a clinical setting, as well as to track the disease

progression and therapy. This would improve the quality of life in people with DS and their families, and would inform clinical trials against AD.

HYPOTHESIS and OBJECTIVES

1. General hypothesis

Assessing Alzheimer's disease-related cognitive decline and diagnosing symptomatic Alzheimer´s disease in people with Down syndrome is challenging due to the coexisting neurodevelopmental intellectual disability. However, the use of adapted neuropsychological tests together with the stratification of the subjects by the individual's level of intellectual disability, and the development of blood and CSF biomarkers will enable earlier and more accurate symptomatic Alzheimer's disease diagnosis and the monitoring of disease progression.

2. Specific hypothesis

1. Clinical progression along the Alzheimer's disease continuum in adults with Down syndrome will be extremely high and age dependent. The Alzheimer's disease-related cognitive decline can be captured with adapted neuropsychological tests.

2. Due to the variability in the severity of the intellectual disability associated with Down syndrome, longitudinal cognitive assessments will have higher diagnostic performance for symptomatic Alzheimer's disease than cross-sectional evaluations.

3. Neurodegeneration biomarkers such as plasma Neurofilament light chain concentrations, in the context of Down syndrome, will have good diagnostic and prognostic performances and will be associated with longitudinal cognitive decline.

4. Synaptic proteins in cerebrospinal fluid will be associated with Alzheimer's disease-related cognitive decline in adults with Down syndrome.

3. Specific objectives

1. To describe the clinical progression and longitudinal cognitive changes with age and along the Alzheimer's disease continuum.

2. To compare the diagnostic performance of cross-sectional and longitudinal neuropsychological assessments for symptomatic Alzheimer´s disease in adults with Down syndrome.

3. To validate the diagnostic and prognostic performance of plasma neurofilament light chain concentrations for symptomatic Alzheimer's disease in adults with Down syndrome, and to analyze their correlation with cognitive performance.

4. To quantify a panel of synaptic biomarkers in cerebrospinal fluid along the Alzheimer's disease continuum in adults with Down syndrome, and to analyze the correlation between synaptic biomarkers and cognitive performance.

METHODS

The specific methods and materials of each work included in this thesis are explained in detail in the corresponding articles. However, a brief outline of the main cohort analyzed, and a brief explanation of the common methodological aspects will be summarized in this section.

1. Study design and setting

All the works included in this thesis are mainly based on the Down Alzheimer Barcelona Neuroimaging Initiative (DABNI) cohort, a prospective longitudinal cohort of adults with Down syndrome with multimodal biomarker studies, which aims to study the natural history of Alzheimer's disease (AD) in Down syndrome (DS). This cohort is recruited and followed at the Alzheimer-Down Unit of the Hospital of Sant Pau and the Catalan Foundation for Down syndrome.

Of note, Study 3 was a multicenter cohort study in collaboration between 6 centers in France, Germany, Spain, the United Kingdom and the United States of America, and study 4 also included non-trisomic controls from the Sant Pau Initiative on Neurodegeneration (SPIN) cohort from the Memory Unit in the Neurology Department of the Hospital of Sant Pau. The details of each of these additional cohorts can be found in the method´s section of Study 3 and, in the case of the SPIN cohort also in Alcolea et al. 2019.[109]

Alzheimer-Down Unit: The Alzheimer-Down Unit was founded in 2012 as a partnership between the Hospital of Sant Pau and the Catalan Foundation for Down syndrome. It has been recognized by the Catalan government as the reference center for all neurological disorders in adults with DS in Catalonia since 2014. The main aim of the Unit is the prevention, early detection, and treatment of AD in adults with DS. To achieve this aim, we developed a pioneer health plan for adults with DS, which includes annual medical and neuropsychological assessments. The DABNI cohort is nested in this health plan and is composed of those individuals that consent to enter research studies. The Alzheimer-Down Unit is located at the

premises of the Hospital of Sant Pau in Barcelona, and it is composed of a multidisciplinary team including neurologists, neuropsychologists, nurses, social workers, and administrative staff, specializing in both, ID and neurodegenerative diseases.

Recruitment started in 2013 and it is still active. Participants in the DABNI cohort are followed annually, and we offer the possibility to repeat imaging and/or CSF studies every two years.

2. Participants

The DABNI cohort includes adults with DS of both sexes over 18 years of age, recruited from the population-based health plan. We include individuals all levels of ID (mild, moderate, severe, and profound).

It is estimated that around 3.500 adults with DS live in Catalonia. The Alzheimer-Down Unit has evaluated almost 1.000 to date. The health plan and the DABNI project have been disseminated in different foundations, residences, occupational centers, and special employment centers, firstly to offer specialized medical attention and, secondly, to expand recruitment and obtain a more representative sample in our studies for the advance in the knowledge of DS and related AD.

For those patients who were not able to physically attend our center, we developed the Domiciliary Alzheimer Visiting in Down Syndrome (DAVIS), initially funded with a competitive grant from the Global Brain Health Institute and then by the Catalan Society of Neurology. This program allowed us to go to different centers in Catalonia to evaluate those individuals with difficulties to come to Barcelona. We have also developed telemedicine visits for those patients with advanced dementia or severe medical problems that could no longer physically attend our center.

3. General procedures

The health plan includes structured annual neurological and neuropsychological assessments by experienced clinicians, but the frequency of the visits is individually adapted according to the patient's specific needs.

All adults with DS attending the Alzheimer-Down Unit are included in the health plan. All individuals are invited to participate in the DABNI cohort, which includes different research projects besides routine health care visits. This initiative includes a multimodal AD biomarkers study to understand the AD natural history in this population. This cohort includes clinical data from the yearly neurological and neuropsychological assessments and optional neuroimaging, plasma, CSF, polisomnography, and genetic biomarker assessments. In addition, other active competitive projects offer the possibility to participate in clinical trial-ready cohorts to speed up recruitment in soon-to-come AD clinical trials and a brain donation program.

Medical visit: Our routine medical/neurological visit consists of a structured anamnesis with the patient and the caregiver, and a physical examination performed by expert neurologists. This visit includes the Cambridge Examination for Mental Disorders of the Elderly with Down syndrome and Others with Disabilities Intellectual (CAMDEX-DS) interview and the Neuropsychiatric Inventory (NPI). We also collect demographic data, clinical and neurological history including epilepsy-associated risk factors, and detailed semiology of epileptic seizures and treatments. We also perform a physical exam including a general neural exam, the Tinetti scale for balance and gait and the Scale for Outcomes in Parkinson's Disease (SCOPA). We finally recommend performing an annual general blood test and we also explain and offer to participate in different research projects.

Neuropsychological assessment: Our main neuropsychological protocol includes the Cambridge Cognitive Examination for Older Adults with Down's Syndrome (CAMCOG-DS) Spanish version[110] and the modified Cued Recall Test (mCRT).[111] At baseline, we establish the individuals' ID level following the DSM-V criteria and we also obtain the IQ assessed with the Kaufman Brief Intelligence Test (K-BIT) Spanish version[112] to further support the ID level classification. However, when there is suspicion of cognitive decline the IQ is not obtained as this intelligence estimation may be biased.

The CAMCOG-DS is a diagnostic test consisting of an objective neuropsychological assessment of the individual with ID which was designed to ensure that most individuals score above the test floor, thus improving the detection of cognitive decline. This cognitive battery comprises 7 subscales for different cognitive domains including: orientation, language (comprehension and expression); memory (new learning, remote, and recent memory); attention; praxis (visoconstrution and motor praxis); abstract thinking; and visoperception. The maximum total score on the CAMCOG-DS is 109 points and a higher score represents a better cognition.

The mCRT is a test following a two-phase paradigma of episodic memory assessment, adapted for individuals with ID. Participants are asked to memorize 12 stimuli presented on 3 cards. There is a learning phase in which items are gradually presented on 4-item carts at a time. Each item is an exemplar of a specific semantic category, and participants are first asked to name and point items and then to associate them with their respective category cues. This phase ensures that participants are familiar with the specific items and have attended to them and their corresponding cues. During the testing phase, participants are asked to recall all items on the list, initially without cues and then with the provision of cues for omitted items during three trials including the free recall first and cued recall thereafter. Different scores can be obtained, first the total Free Inmediate Recall (FIR) where the free recall of the three trials are summed, as well the Total Inmediate Recall (TIR) which comprises de sum of the free recall and the cued recall. Intrusive errors can be also registered.

Diagnostic process: After these two independent visits, neurologists and neuropsychologists clinically classify independently the subjects into 4 possible diagnostic categories:

- **Asymptomatic:** when there is no clinical or neuropsychological suspicion of AD
- **Prodromal AD:** when there is suspicion of AD, but symptoms do not fulfill the criteria for dementia
- **AD dementia:** when there is full-blown AD dementia with cognitive decline and impairment in activities of daily living
- **Uncertain/ non- degenerative neurocognitive disorder:** When the person has a medical, pharmacological, or psychiatric condition interfering with cognition or activities of daily living, but no suspicion of neurodegenerative origin. Of note, in some instances, these conditions are treatable and reversible and individuals can change their clinical classification to one of the other three categories.

It is important to note that we the diagnostic process is a sequential one to avoid circularity in the research studies. After the independent visits, neurologists and neuropsychologists establish a “Neurologist diagnosis” and a “Neuropsychologist diagnosis” separately, blind to each other’s visit and blind to biomarker results. After these independent diagnoses, there is a consensus clinical meeting between both professionals to determine the “Consensus clinical diagnosis” based on the whole clinical information. Finally, the diagnosis is revisited including biomarkers information and/or clinical longitudinal follow-up information to establish the “Definitive diagnosis” of a given visit. This scheme was designed to avoid circularity in the diagnostic and prognostic research studies including cognitive and biomarker measures.

We define progression along the AD continuum when there is a change in the clinical and cognitive status after excluding other medical and psychosocial conditions that might justify the changes. We consider progression from asymptomatic to prodromal AD when there are relevant cognitive or behavioral changes, but there is no additional interference in daily living activities, and from prodromal to AD dementia when the cognitive and behavioral changes do interfere with the personal premorbid autonomy of patients.

CSF collection and biomarker assessment: We perform a lumbar puncture to collect 15-20 mL CSF sample. CSF samples are collected and processed in polypropylene tubes following international recommendations.[113] The sample is transferred to our laboratory where samples are processed and aliquoted within the first two hours after the lumbar puncture. Aliquots are stored at -80 ºC and are not thawed before analyses. Core AD biomarkers (Ab1-42, t-Tau, and p-Tau) are routinely measured in all participants. We use commercially available fully automated immunoassays to determine levels of CSF AB_{1-40}, AB_{1-42}, NFL, total tau, and p-tau at threonine residue 181 (Lumipulse AB_{1-40}, AB_{1-42}, NFL, total tau G, p-tau 181, Fujirebio-Europe, NFL Simoa Quanterix, MA, USA).

Blood extraction and analysis: Blood extraction is performed at the time of lumbar puncture or during the routine visit in those participants who do not consent to lumbar puncture. All samples are transferred to our laboratory where they are centrifuged and aliquoted within 2 hours after extraction and stored at -80 ºC until they need to be analyzed. Fasting is not mandatory before the extraction, but the time from the last meal to the blood extraction is recorded. Plasma Aβ1–40, Aβ1–42, t-tau, and NfL concentrations are locally measured using the ultrasensitive SIMOA assay.[109]

4. Statistical analysis

All statistical analyses were performed in R (https://www.r-projrct.org/) or STATA statistical softwares.

Some figures included in this thesis were created with BioRender.com

5. Ethical aspects

All studies were conducted at the Alzheimer-Down Unit in strict accordance with international ethical guidelines for medical research in humans following standards contained in the Declaration of Helsinki and Spanish law. Before the start of any study, all protocols, the information given to the subjects as well as the informed consent model used, were approved by the Sant Pau Research Ethics Committees.

Before including any subject in the study, the investigator gave detailed information to the participant and they legally authorized representative of the objectives, methods, potential risks, or any inconvenience it may cause. Moreover, all participants could ask for extra information or clarification whenever needed, and they were able to abandon the study at any point for any reason.

All participants or their legally authorized representative were asked for their consent to the acquisition, analysis, and storage of biological samples. They are also informed about the possibility of sharing anonymized information and/or biological samples with other researchers. Confidentiality was guaranteed in accordance with current Spanish legislation (LOPD 3/2018).

PUBLICATIONS

Longitudinal Clinical and Cognitive Changes Along the Alzheimer Disease Continuum in Down Syndrome

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ABSTRACT

Importance: Alzheimer disease (AD) is the main medical problem in adults with Down syndrome (DS). However, the associations of age, intellectual disability (ID), and clinical status with progression and longitudinal cognitive decline have not been established.

Objective: To examine clinical progression along the AD continuum and its related cognitive decline and to explore the presence of practice effects and floor effects with repeated assessments.

Design, setting, and participants: This is a single-center cohort study of adults (aged >18 years) with DS with different ID levels and at least 6 months of follow-up between November 2012 and December 2021. The data are from a population-based health plan designed to screen for AD in adults with DS in Catalonia, Spain. Individuals were classified as being asymptomatic, having prodromal AD, or having AD dementia.

Exposures: Neurological and neuropsychological assessments.

Main outcomes and measures: The main outcome was clinical change along the AD continuum. Cognitive decline was measured by the Cambridge Cognitive Examination for Older Adults With Down Syndrome and the modified Cued Recall Test.

Results: A total of 632 adults with DS (mean [SD] age, 42.6 [11.4] years; 292 women [46.2%]) with 2847 evaluations (mean [SD] follow-up, 28.8 [18.7] months) were assessed. At baseline, there were 436 asymptomatic individuals, 69 patients with prodromal AD, and 127 with AD dementia. After 5 years of follow-up, 17.1% (95%CI, 12.5%-21.5%) of asymptomatic individuals progressed to symptomatic AD in an age-dependent manner (0.6%[95%CI, 0%-1.8%] for age <40 years; 21.1% [95%CI, 8.0%-32.5%] for age 40-44 years; 41.4%[95%CI, 23.1%-55.3%] for age 45-49 years; 57.5% [95%CI, 38.2%-70.8%] for age50 years; P < .001), and 94.1%(95%CI, 84.6%-98.0%) of patients with prodromal AD progressed to dementia with no age dependency. Cognitive decline in the older individuals was most common among those who progressed to symptomatic AD and symptomatic individuals themselves. Importantly, individuals with mild and moderate ID had no differences in longitudinal cognitive decline despite having different performance at baseline. This study also found practice and floor effects, which obscured the assessment of longitudinal cognitive decline.

Conclusions and relevance: This study found an association between the development of symptomatic AD and a high risk of progressive cognitive decline among patients with DS. These results support the need for population health plans to screen for AD-related cognitive decline from the fourth decade of life and provide important longitudinal data to inform clinical trials in adults with DS to prevent AD.

INTRODUCTION

Down syndrome (DS) is the most frequent cause of intellectual disability (ID) of genetic origin, affecting 5.8 million peopleworldwide.1 In adults with DS, Alzheimer disease (AD) is the main medical problem and main cause of death.2 Indeed, the AD pathological hallmarks are universal by age 40 years,3 and the dementia prevalence increases exponentially thereafter,4-7 with a cumulative incidence of more than 95%in the seventh decade. This is mainly

owing to the presence of an extra copy of the amyloid-β precursor protein gene, which is coded in chromosome 21.8 Consequently, DS is considered a genetic form of dementia, similar to autosomal dominant AD (ADAD).2,6,9 Importantly, the clinical and AD biomarker changes are strikingly similar in both populations.6

ID is defined as a condition characterized by substantial limitations in intellectual functioning, as well as in adaptive behavior. The premorbid ID associated with DS can overshadow AD-related cognitive decline, and it also explains the floor effects found in traditional neuropsychological tests used in general population. Furthermore, health professionals from the general population do not feel confident when attending people with DS.10 For these reasons, people with DS require adapted tests to assess cognitive performance, as well as specific medical care.11 Recent studies12 show that adapted neuropsychological tests are useful for the diagnosis of prodromal and AD dementia at a cross-section when stratifying by the level of ID. Some tests, such as the modified Cued Recall Test (mCRT), are also useful to capture early AD-associated cognitive decline in asymptomatic adults with DS.13,14 However, given differences in premorbid ID level, clinical guidelines have emphasized the need for tracking within-person changes over time to detect AD-related cognitive decline.15 There are, however, only a few studies16-19 that have assessed longitudinal AD-related cognitive decline. These studies2,20,21 have shown early declines in episodic memory and executive function, but most of them had a small sample size and/or short duration of follow-up, and none of them stratified the findings by ID or age ranges. Finally, floor effects and practice effects can obscure the measurement of cognitive decline, thus affecting cognitive end points in AD clinical trials in this population.22 These effects have not been assessed in the AD continuum in DS.

This study evaluated the largest single-center, population-based longitudinal cohort of adults with DS to examine the clinical and the cognitive changes along the AD continuum. We also explored the presence of practice and floor effects.

METHODS

Study Design, Setting, and Population: This is a single-center, prospective, longitudinal, cohort study of adults with DS recruited at the Alzheimer-Down Unit from the Catalan Down Syndrome Foundation and Hospital of Sant Pau, in Barcelona, Spain. We recruited participants of both sexes aged 18 years or older from a population-based health plan designed to screen for AD in adults with DS in Catalonia. This health plan includes structured semi-annual or annual neurological and neuropsychological assessments by experienced clinicians. We included individuals with all levels of ID and a minimum follow-up of 6 months. Individuals with severe and profound ID were excluded in the cognitive analyses, as these individuals perform at floor scores.12 eFigure 1 in the Supplement shows the study flowchart.

The study was approved by the Sant Pau Research Ethics Committees, following the standards for medical research in humans recommended by the Declaration of Helsinki.23 All participants or their legally authorized representative gave written informed consent. Confidentiality was guaranteed in accordance with current Spanish legislation. This report follows the Streng-

thening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline.

Study Outcomes: The study procedures included a medical examination with the participant and main caregiver, as well as a neuropsychological assessment whenever possible.12,24 For further details of the diagnostic process, see the eAppendix in the Supplement.

The neuropsychological assessment included the Cambridge Cognitive Examination for Older Adults With Down Syndrome (CAMCOG-DS) Spanish version25 and the mCRT.13 ID was categorized as mild, moderate, severe, or profound according to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition, and on the basis of caregivers' reports of the individuals' best-ever level of functioning and the score of the Kaufman Brief Intelligence Test Spanish version.26

The CAMCOG-DS is an adapted cognitive battery with a maximum score of 109. The mCRT is an adapted test to assess free and cued episodic memory, and its maximum score is 36.13 In the main text we show the free immediate recall (FIR) score. In both tests, higher scores indicate better cognition. We defined practice effects as any change or improvement that results from repetition of task items, and floor effects as the situation in which a large proportion of participants perform very poorly on a task.27

Participants were classified clinically into 4 groups in a consensus meeting between the neurologist and neuropsychologist after independent visits: (1) asymptomatic (ie, no clinical or neuropsychological suspicion of AD), (2) prodromal AD (ie, suspicion of AD, but symptoms did not fulfill criteria for dementia), (3) AD dementia (ie, full-blown AD dementia), and (4) uncertain or nondegenerative neurocognitive disorder (ie, when there were medical, pharmacological, or psychiatric condition interfering with cognition or daily living activities, but no suspicion of neurodegenerative origin). Of note, in some instances, these conditions were treatable and reversible, and individuals were classified in 1 of the other 3 categories at follow-up visits. We excluded all the visits with an uncertain diagnosis. For the prognostic evaluation, asymptomatic participants and those with prodromal AD were subsequently classified as progressors when there was a change in the clinical diagnosis along the AD continuum. Participants who remained in the same AD diagnostic category were classified as nonprogressors.

To estimate longitudinal cognitive decline in the different clinical groups, we included all data points from baseline for each category. For prodromal AD and AD dementia, we also included the data points of progressors after the change in diagnostic category.

Statistical Analysis: To assess the descriptive statistics for the baseline data, we performed analysis of variance tests for numerical variables and $\chi 2$ tests of independence for categorical variables. Analyses were performed in R statistical software version 3.6.3 (R Project for Statistical Computing).

To assess clinical progression, we used Kaplan-Meier curves in the whole sample and in different age ranges, in the latter followed by log-rank tests. We used linear mixed-effects models (LME) in the R lme4 package to model the longitudinal cognitive changes as a function of age in individuals with mild and moderate ID separately,

including linear and quadratic (when significant) age terms as fixed effects and participant-specific intercepts and slopes as random factors. We tested the interaction term between clinical diagnosis and time. Both raw cognitive scores and cognitive annualized change (follow-up – baseline / years between both time points) were used separately as dependent variables in these analyses. We also assessed the longitudinal cognitive decline in each clinical diagnostic group by applying an LME with an interaction term between diagnostic group and years of follow-up using participant-specific intercepts and slopes as random factors. We finally divided the sample into age ranges and applied an LME with an interaction term between the age intervals and years of follow-up with random intercept and slope for each individual. To assess the practice and floor effects, we plotted the mean cognitive scores at each year of follow-up (for each age range and the different clinical groups, respectively). For the latter, we also modeled the annualized change with respect to the baseline performance with generalized additive models calculated with the Rmgcv package with random effects (intercept and slope) for each participant. All statistical analyses were performed using 2-sided tests with a level of significance at $P < .05$.

RESULTS

Study Population: eFigure 1 in the Supplement shows the study flowchart. From November 2012 to December 2021, we included 632 adults with DS (mean [SD] age, 42.6 [11.4] years; 292 women [46.2%]) who had longitudinal clinical follow-up visits. Of these, 433 (68.5%) had longitudinal neuropsychological assessments. The Table displays baseline demographic and cognitive data by clinical diagnosis for the whole sample and for the subgroup with longitudinal cognitive assessments: 436 individuals (69.0%) were asymptomatic, 69 (10.9%) had prodromal AD, and 127 (20.1%) had AD dementia. As expected, asymptomatic individuals were younger and had higher cognitive scores than patients with prodromal AD and AD dementia (Table). There were no significant differences in sex distribution, but there were differences in ID across the clinical groups; there was a higher proportion of individuals with moderate ID in all groups. The mean (SD) follow-up in the whole cohort was 28.8 (18.7) months. The follow-up interval was longer in asymptomatic individuals (mean [SD], 31.0 [18.8] months) than in those with prodromal AD (mean [SD], 18.2 [18.8] months) or those with AD dementia (mean [SD], 26.9 [16.2] months) (Table).

Clinical Progression: Figure 1 shows the Kaplan-Meier curves for the clinical progression in the whole sample and for the different age ranges in asymptomatic individuals and those with prodromal AD separately. Overall, after 5 years of follow-up, 17.1% (95%CI, 12.5%-21.5%) of the asymptomatic individuals had progressed to symptomatic AD (Figure 1A), and 94.1% (95%CI, 84.6%-98.0%) of the prodromal group had progressed to dementia (Figure 1B). eTable 1 in the Supplement shows the progression rates at different follow-up times in the different age ranges. The clinical progression in asymptomatic individuals showed a clear age dependency: only 0.6%(95%CI, 0.0%-1.8%) of individuals younger than 40 years in the asymptomatic group progressed to symptomatic AD after 5 years of follow-up, whereas 57.5%(95%CI, 38.2%-70.8%) of those older than 50 years did (corresponding percen-

	Whole sample (N= 632)			
	Asymptomatic	Prodromal	Dementia	P Value
	n=436	n=69	n=127	
Female (%)	199 (45.6)	32 (46.4)	61 (48.0)	.893
Age mean (SD)	38.0 (10.4)	50.9 (4.9)	53.7 (5.6)	<.001
ID (%)				<.001
mild	101 (23.2)	10 (14.5)	10 (7.9)	
moderate	206 (47.2)	46 (62.3)	67 (52.8)	
severe/profound	29.3%	24.3%	39.0%	
CAMCOG-DS mean (se)	NA	NA	NA	NA
mCRT FIR mean (se)	NA	NA	NA	NA
Follow Up months mean (SD)	31.0 (18.8)	18.2 (18.8)	26.9 (16.2)	<.001
	Cognition Sample (N= 433)			
	Asymptomatic	Prodromal	Dementia	P Value
	n=304	n=53	n=76	
Female (%)	149 (49.0)	26 (49.1)	40 (52.6)	.849
Age mean (SD)	36.9 (9.6)	51.0 (5.1)	53.0 (5.6)	<.001
ID (%)				<.001
mild	100 (22.9)	10 (18.9)	10 (13.2)	
moderate	204 (67.1)	43 (81.1)	66 (88.8)	
severe/profound	NA	NA	NA	
CAMCOG-DS mean (se)	75.6 (16.0)	47.5 (18.7)	47.1 (17.1)	<.001
mCRT FIR mean (se)	19.5 (6.17)	8.82 (5.93)	4.09 (4.57)	<.001
Follow Up months mean (SD)	32.5 (18.5)	18.3 (19.2)	27.0 (15.8)	<.001

Table. Demographic and Cognitive Variables by Clinical Diagnosis at Baseline for the Whole Sample and the Cognitive Analysis Subsample

tages were 21.1%[95%CI, 8.0%-32.5%] for those aged 40-44 years and 41.4%[95%CI, 23.1%-55.3%] for those aged 45-49 years; P < .001) (Figure 1C). Progression to AD dementia in patients with prodromal AD, on the other hand, was almost universal after 5 years of follow-up, and, importantly, it did not show such an age dependency. eFigure 2 in the Supplement shows these results stratified by ID (eFigure 2A and 2B in the Supplement) and by sex (eFigure 2C and 2D in the Supplement). There were no significant differences by ID or between men and women in asymptomatic individuals. However, women with prodromal AD had a faster progression than men ($\chi 2$ 1 = 4.3; P = .04).

Longitudinal Cognitive Outcomes: We next analyzed the longitudinal cognitive data. We first studied the percentage of individuals who were able to complete the cognitive tests at the different follow-up visits by clinical diagnosis and level of ID (eFigure 3 in the Supplement). During the follow-up period, many individuals in symptomatic stages were not able to complete the tests.

Figure 2 shows the changes in the CAMCOG-DS and mCRT performance with age in adults with DS (individuals with mild and moderate levels of ID are analyzed separately). As expected, the scores were higher for individuals with mild ID than those with moderate ID at all ages (mean [SE] difference, −19.24 [1.68] for CAMCOG-DS and −4.06 [0.55] for mCRT; P< .001) (Figures 2A and 2B), but the interaction term of ID with age was not significant, suggesting that there were no differences in the trajectories between the ID groups. Similarly, and importantly, the annualized change did not differ between individuals with mild and moderate ID in either test (Figures 2C and

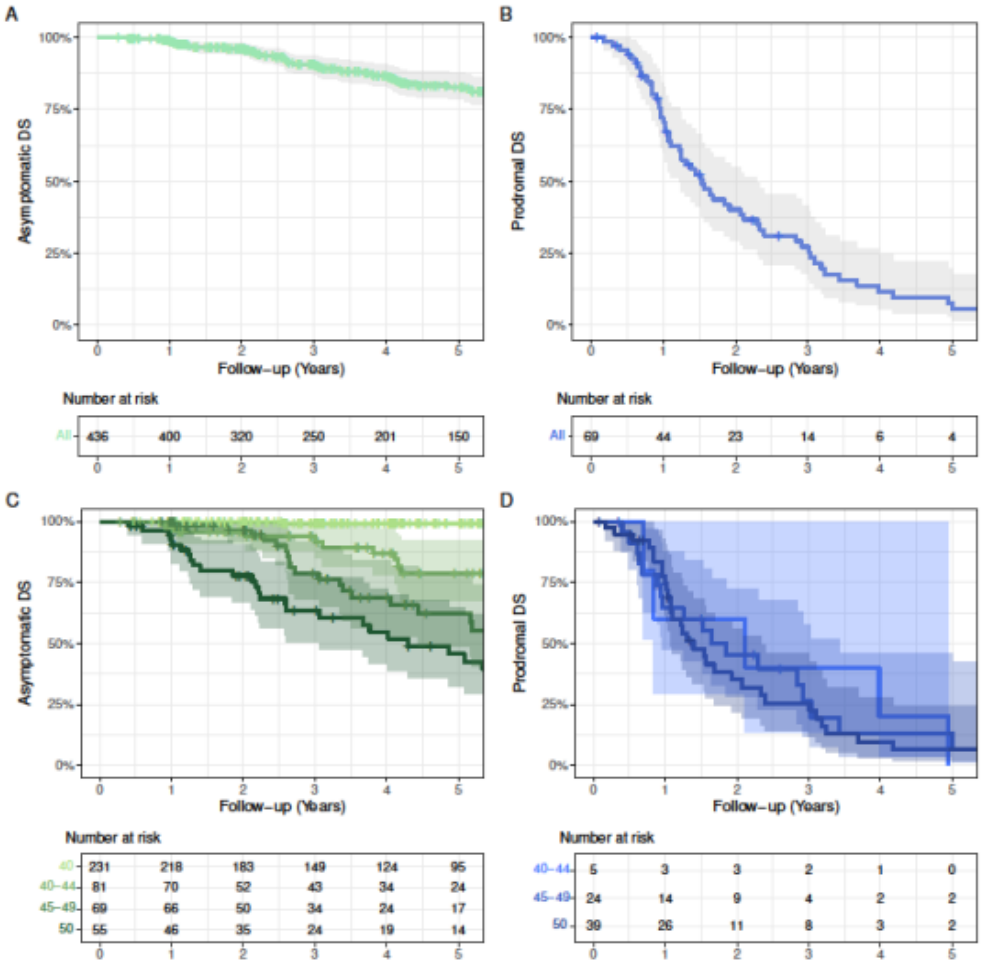


Figure 1. Clinical Progression of Asymptomatic Individuals and Those With Prodromal Alzheimer Disease (AD) Among Adults With Down Syndrome (DS)

Kaplan-Meier curves are shown for all asymptomatic individuals (A), all those with prodromal AD (B), asymptomatic individuals by age range (C), and those with prodromal AD by age range (D). Shaded areas indicate 95% CIs.

2D), but showed a similar cognitive decline with age (mean [SE] score difference of −0.15 [0.04] points per year on the CAMCOG-DS and −0.06 [0.02] points per year on the mCRT). Of note, the LME analyses showed a significant age quadratic term in the model for the raw scores for both tests and increases in the longitudinal performance in younger individuals, suggesting the presence of practice effects (see eFigure 4 in the Supplement for CRT total immediate recall results). Sex was not associated with cognitive performance with age, or in the annual change, except for the raw CAMCOG-DS in the mild ID group, where women had a quadratic trajectory different than that of men (β [SE], −360.68 [32.36] for CAMCOG-DS and 85.90 [11.71] for mCRT; P = .006), although they did not decline faster than men.

To assess the presence of practice effects, we first analyzed the longitudinal cognitive trajectories in the asymptomatic individuals in the different age ranges. These analyses showed the presence of practice effects during the first 2 years in asymptomatic individuals (mainly in the mCRT FIR), either as early increases in the cognitive tests with subsequent stabilization, or as early stability with subsequent decline (Figures 3A and 3B).We, therefore, estimated the trajectories for the CAMCOG-DS and mCRT FIR scores for the first 2 years of follow-up and those beyond separately (Figures 3C and 3D) to estimate the cognitive decline when there are no practice effects (those after 2 years of follow-up).We finally estimated the longitudinal trajectory of change for the 2 tests in the different age ranges (Figures 3E and 3F).

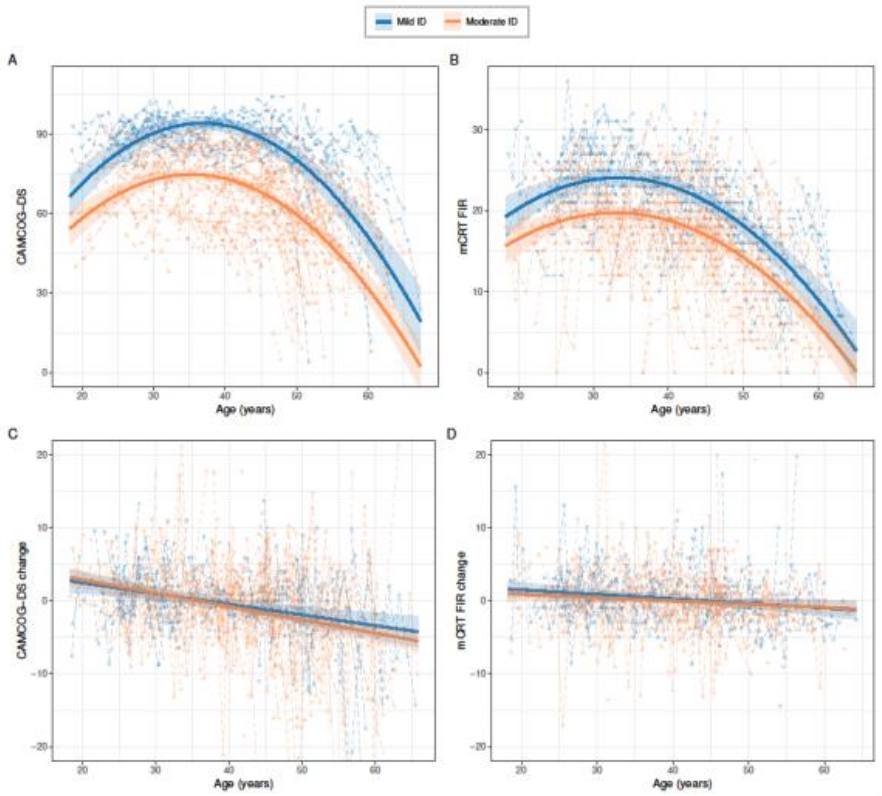


Figure 2. Changes in Cambridge Cognitive Examination for Older Adults With Down Syndrome (CAMCOG-DS) and Modified Cued Recall Test (mCRT) Free Immediate Recall (FIR) Scores With Age in Individuals With Mild and Moderate Levels of Intellectual Disability (ID) Separately

Graphs show quadratic association between age and CAMCOG-DS raw scores (A) and mCRT FIR raw scores (B) in patients with mild (blue dots) and moderate (orange dots) ID. Panels C and D show the association between the annualized cognitive change and age by ID in CAMCOG-DS (C) and mCRT FIR (D).

Practice effects (apparent as longitudinal improvement in the cognitive tests) were clear in younger individuals (see eFigure 5 in the Supplement for CRT total immediate recall results). In the stratified analyses by sex, there were no significant differences, except for the trajectory in CAMCOG-DS after 2 years of follow-up in the age group of 40 to 49 years, where women declined faster than men (mean [SE], 2.90 [1.36] points per year; P = .04).

Figure 4 shows the longitudinal cognitive changes in the different clinical groups in the combined sample of adults with mild and moderate ID (eFigures 6 and 7 in Supplement show these changes stratified by the level of ID). As expected, there was a progressive decline in CAMCOG-DS and mCRT FIR scores along the AD continuum (for the CAMCOG-DS: asymptomatic individuals vs asymptomatic progressor individuals, mean [SE], −2.00 [0.59] points per year; asymptomatic individuals vs those with prodromal AD, mean [SE], −6.29 [0.59] points per year; asymptomatic individuals vs those with AD dementia, mean [SE], −8.19 [0.71] points per year; for the mCRT: asymptomatic individuals vs asymptomatic progressor individuals, mean [SE], −1.72 [0.17] points per year; asymptomatic individuals vs those with prodromal AD, mean [SE], −1.71 [0.24] points per year; asymptomatic individuals vs those with AD dementia, mean [SE], −1.69 [0.34] points per year; P < .001 for all comparisons). However, visual analyses of the trajectories suggested early floor effects for the mCRT FIR in symptomatic individuals and a wider dynamic range for the CAMCOG-DS. To further assess the floor effects (dynamic range in the different groups) of the tests, we plotted the annualized longitudinal change in the score with the baseline performance (Figures 4E and 4F).

There was an increasing decline in CAMCOG-DS scores along the AD continuum,

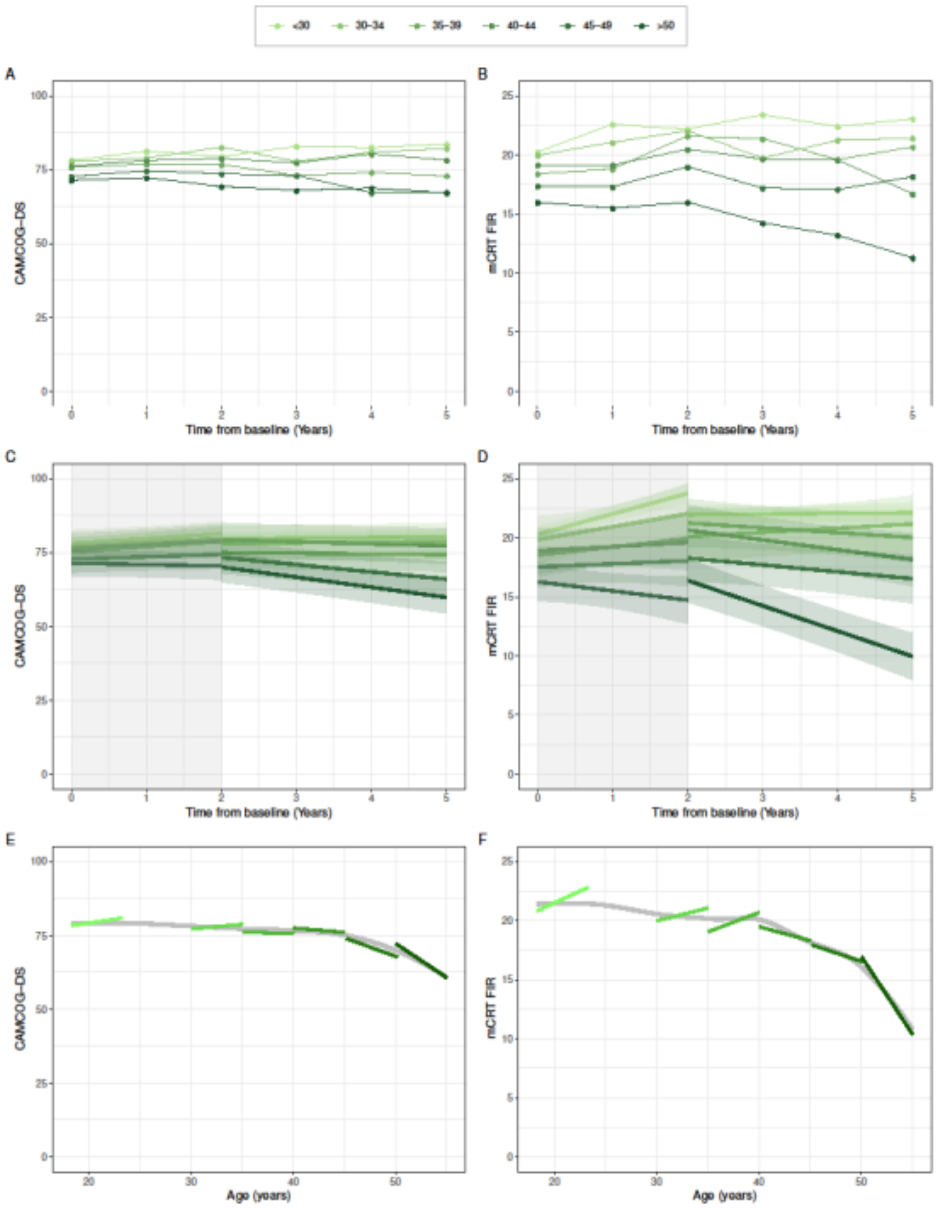
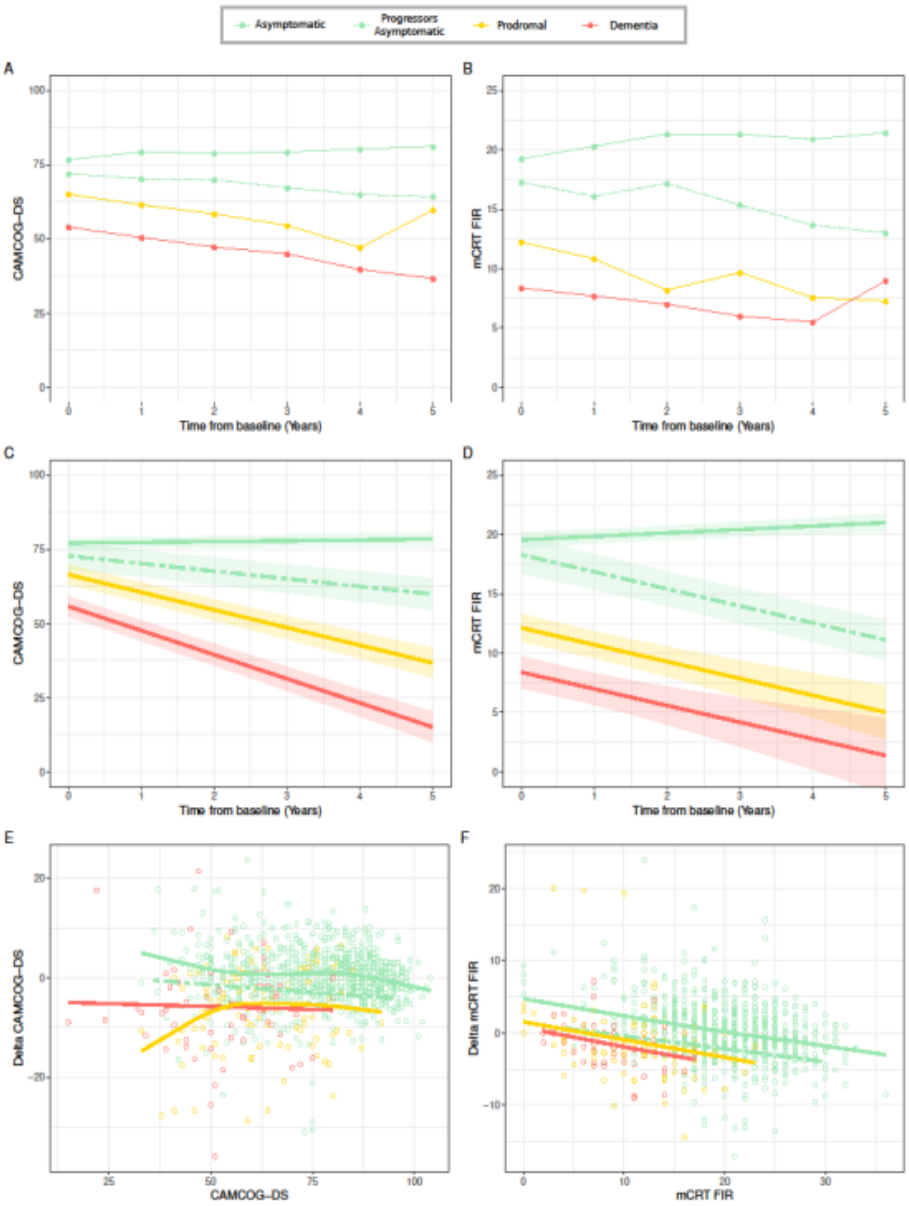


Figure 3. Cognitive Trajectories by Age Ranges in Asymptomatic Participants With Down Syndrome (DS) Showing Learning Effects in Younger Individuals During the First 2 Years of Follow-up

Panels A and B show Cambridge Cognitive Examination for Older Adults With Down Syndrome (CAMCOG-DS) (A) and modified Cued Recall Test (mCRT) free immediate recall (FIR) (B) raw scores by age ranges along 5 years of follow-up. Panels C and D show CAMCOG-DS (C) and mCRT FIR (D) estimated slopes for the cognitive trajectories during the first 2 years of follow-up and beyond calculated separately by age ranges during the follow-up. Panels E and F show that CAMCOG-DS (E) and mCRT FIR (F) practice effects were seen several years after a decline in (baseline) cognitive scores with age was observed.

but this decline was independent of the baseline scores. However, in themCRT, although there was a similar decline along the AD continuum, the longitudinal decline was dependent on the baseline scores, and those with scores lower than 10 to 15 did not show longitudinal decline (ie, were at floor effects of the test; see eFigure 8 in the Supplement for the mCRT total immediate recall results). When including the sex to the model, women had a faster cognitive decline than men on the mCRT (mean [SE], 0.11 [0.05] points per year; P = .04) but not the CAMCOG-DS; nonetheless, when we stratified by clinical diagnosis, this effect disappeared.

eTables 2 and 3 in the Supplement show the annualized change for the CAMCOG-DS and CRT scores in the different clinical groups and for the different age ranges in asymptomatic individuals. There was significant decline for the CAMCOG-DS after age 45 (mean [SE], −1.25 [0.31] points per



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year; P = .03) and after age 40 for the mCRT FIR (mean [SE], −0.23 [0.13] points per year; P = .03).

DISCUSSION

To our knowledge, this is the largest population-based cohort study of adults with DS with longitudinal clinical and neuropsychological assessments. The large sample size enabled us to estimate for the first time both the risks of progression along the AD continuum at different ages ranges and different follow-up times and the longitudinal cognitive changes by level of ID and by clinical group. We found that, although the level of ID must be considered when using neuropsychological tests for diagnosis, it might not be necessary to monitor longitudinal decline. We also showed for the first time practice effects and floor effects that might impact cognitive end points in clinical trials.

Longitudinal progression along the AD continuum showed a clear age dependency in our study in asymptomatic individuals. Progression was rare before age 40 years but was seen in 57.5% of those older than 50 years after 5 years of follow-up. This age dependency was not seen in patients with prodromal AD, who universally progressed to AD dementia after 5 years. The risk for progression along the AD continuum is very similar to that described in ADAD, now estimated in both populations to be more than 95%in longitudinal studies.2,5,7 Data from general population in sporadic AD are more variable, especially because of the study setting and selection criteria (eg, population-based vs convenience cohorts and different mean ages) and different definitions of progression. Petersen28 reported an overall annual progression from mild cognitive impairment to AD of 8% to 15% when biomarkers are not evaluated. However, when AD biomarkers are considered, the risk for those with positive biomarkers increases substantially. For example, a previous study29 found a 38% (95%CI, 21%-59%) risk of progression from mild cognitive impairment to dementia in those patients with positive amyloid and neurodegeneration biomarkers. Age, as in our study, is another critical factor to consider, especially in cognitively healthy individuals. Progression rates in cognitively healthy euploid individuals increase with age. For example, Roberts et al30 found a 1-year risk of progression to mild cognitive impairment of 3.59%in those aged 70 to 74 years, 4.49% in those aged 75 to 79 years, 8.63%in those aged 80 to 84 years, and 13.5%in those aged 85 to 89 years. In short, the main difference between the progression rates and those of the general population are the age at which symptom onset manifest, which is 40 years younger in DS, and the fact that in DS, all patients have (at least) preclinical AD by definition,2,9 whereas in the general population the underlying causes of cognitive decline are more heterogeneous.

The cognitive substudy has 4 main findings. First, it confirms the feasibility of performing long-term longitudinal neuropsychological assessments in asymptomatic individuals with DS and in a subset of symptomatic individuals. Second, individuals with mild and moderate ID had similar rates of longitudinal cognitive decline, despite the different offset at all ages. Third, this study found practice effects, most prominently in the episodic memory test. The practice effects obscured the assessment of cognitive decline. Indeed, the observed longitudinal cognitive changes are the net effect of practice effects minus longitudinal cognitive decline. Fourth, we also found floor effects in the episodic memory test, but not in the CAMCOG-DS. The mCRT is, thus, very sensitive to early changes in preclinical and prodromal AD in DS but has clear floor effects (and less applicability) in symptomatic stages to monitor decline. The CAMCOG-DS, although less sensitive to change in preclinical AD, has a better dynamic range in symptomatic individuals and, thus, is better suited for the monitoring of AD progression in symptomatic individuals.

Our findings have several implications for public health and clinical practice. Although the risk of developing dementia before the age of 40 years is low,31 cognitive decline (once practice effects are accounted for) starts earlier in individuals with DS (10-15 years before the median diagnosis of prodromal AD),6,7 in agreement with previous work32-34 showing that longitudinal AD-related cognitive decline starts in the fourth decade in people with DS. This temporality of cognitive de-

cline is similar to that described in ADAD, starting with episodic memory decline in the preclinical stage.2,18,35,36 Population-based health plans to screen for AD should, therefore, start at approximately age 35 years to detect those individuals at higher risk to progress to dementia. The clinical identification of this high-risk population, most likely in combination with biomarkers, will give people with DS and their families and caregivers the opportunity of an early diagnosis, professional counseling, and treatment.

Our findings also might inform the design of clinical trials. Individuals with DS constitute the largest population of those genetically determined AD and, thus, probably constitute the best population in which to perform preventive clinical trials, even though adults with DS have been largely excluded from AD clinical trials.2 Our results underscore this missed opportunity. First, the extremely high progression rates to symptomatic AD confirm that a preventive trial would have high statistical power. Second, we confirm that it is possible to capture and monitor cognitive decline due to AD in this population (in individuals with mild or moderate levels of ID) for the duration of a preventive trial. Importantly, because there are no differences in longitudinal cognitive decline between those with mild and moderate ID, we propose that it might not be necessary to stratify by the level of ID to monitor disease progression in the cognitive end points (as opposed to the use of cross-sectional neuropsychological tests for AD diagnosis),12 a result that would undoubtedly facilitate recruitment and power. The practice effects and floor effects must also be considered in the analyses. Practice effects should be considered and modeled, especially when in the context of a trial recruiting participants de novo and from longitudinal cohorts. However, they can increase the dynamic range of the tests and, therefore, their power to detect a response to treatment, especially in the context of short trials. This might explain some of the divergent effects in clinical trials between the cognitive trajectories of the placebo group and historical longitudinal cohorts in sporadic AD and ADAD,37 irrespective of the randomization and potential treatment effect.

Strengths and Limitations: The main strengths of this study are the large sample size and that it comes from a well-characterized large cohort of adults with DS. Thus, we have objective and reliable longitudinal cognitive data obtained with an extensive neuropsychological evaluation.

This study also has limitations. First, it is a single-center study and, thus, needs to be replicated in other cohorts to confirm the generalizability of our results. Second, the follow-up might have been insufficient to fully capture the risk in the younger individuals. Third, individuals with severe and profound ID could not be included in the cognitive analyses. Fourth, we did not analyze the impact of the different biomarkers or APOE on progression or cognitive decline and we based all the diagnosis and progression on clinical criteria. Future studies should incorporate biomarkers, especially plasma biomarkers, because of their wider availability and lower costs, to enable better risk stratification of the individuals. We also think that there is a need to develop cognitive tools to assess AD-related cognitive decline in this population suitable for severe or profound ID levels.

Conclusions: In summary, this study found a very high risk of developing symptomatic AD associated with progressive cognitive

decline among adults with DS. These findings support the need for population health plans to screen for AD-related cognitive decline and underscore the imperative and the opportunity to conduct AD preventive clinical trials in adults with DS.

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Cross-sectional versus longitudinal cognitive assessments for the diagnosis of symptomatic Alzheimer's disease in adults with Down syndrome

Submitted. Under review

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SUMMARY

Background: Down syndrome (DS) is a form of genetically determined Alzheimer's disease (AD). However, clinical diagnosis of symptomatic AD is difficult due to the premorbid intellectual disability (ID) and the lack of diagnostic criteria and population-based norms.

Objective: To compare the diagnostic performance of cross-sectional versus longitudinal neuropsychological assessments to detect prodromal AD and AD dementia in adults with DS.

Methods: Single-center longitudinal cohort study of adults with DS recruited from November 2012 to October 2022. We included adults with DS with at least one neuropsychological assessment with mild or moderate levels of ID. Participants were classified based on the physician criteria blind to the neuropsychological assessment to avoid circularity into 3 clinical groups: asymptomatic DS (aDS), prodromal AD (pDS) or AD dementia (dDS). The neuropsychological assessment included the Cambridge Cognitive Examination for Older Adults with Down's syndrome (CAMCOG-DS) and the modified Cued Recall Test (mCRT). We performed receiver operating characteristic curve (ROC) analyses to compare the Areas Under the Curve (AUC) of the CAMCOG-DS and mCRT at baseline and the longitudinal change at different follow-ups. We derived cut-offs for the diagnosis of prodromal AD and AD dementia from the ROC analyses and assessed the stability of these cut-offs with disease progression of symptomatic AD patients. Finally, we derived population-based normative data from young adults with DS.

Results: A total of 589 adults with DS (with 2119 cognitive evaluations) were included with a mean follow-up of 23.7 months. At baseline, there were 440 aDS, 63 pDS and 86 dDS. aDS were younger than pDS and dDS (mean age [SD] 37.05 [9.59], 51.55 [4.78], 52.77 [5.55] respectively; p=0.00). CAMCOG-DS and mCRT total immediate recall showed good diagnostic performances to distinguish between aDS from pDS and dDS at baseline (all AUCs >0.80, except for the CAMCOG-DS to detect pDS, AUC=0.71). Importantly, baseline AUC were higher than the 1-year longitudinal cognitive change (all AUCs<=0.72), but the AUC for the longitudinal cognitive change progressively increased with longer follow-ups. The cut-offs derived from the ROC analyses were greatly influenced by the severity of the symptomatic AD cases included. Finally, we propose cut-offs based on population norms derived from the cognitive performance of young aDS individuals.

Discussion: Contrary to current diagnostic recommendations, baseline cognitive assessment has a higher diagnostic performance than 1-year intra-individual cognitive decline.

INTRODUCTION

Down syndrome (DS) is the most frequent cause of intellectual disability (ID) of genetic origin,[1] affecting 5.8 million people worldwide.[2-4] Alzheimer disease (AD) neuropathological hallmarks are universal by the age of 40 in adults with DS,[5] and dementia incidence (and prevalence) increases exponentially with age from the fifth decade of life[3,4,6-8] with a cumulative incidence in excess of 90% in the seventh decade.[3,7,9] This is mainly due to the presence of an extra copy of the amyloid β precursor protein (APP) gene, which is coded in chromosome 21[3,9,11]. Consequently, DS is considered a genetic form of dementia, similar to autosomal dominant Alzheimer disease (ADAD),[64,114,115] and both populations show strikingly similar clinical and AD biomarker changes.[3,12]

The diagnosis of prodromal AD and AD dementia in individuals with DS is a challenge due to the premorbid ID and the lack of diagnostic criteria.[13] In the general population, the clinical diagnosis of prodromal AD (or mild cognitive impairment) or AD dementia requires a change in cognition based on neuropsychological tests relative to population norms.[14] In people with DS, however, most test

tests commonly used in the general population are of limited use due to many individuals scoring at floor.[15] From a neuropsychological perspective, the variable levels of ID within and between individuals with DS had impeded the development of validated cut-offs.[16,17] In fact, the National Task Group Consensus Recommendations emphasized the need tracking cognition over time in longitudinal assessments for the diagnosis of dementia in individuals with ID.[18]

Nonetheless, very recent studies have showed that adapted neuropsychological tests such as the Cambridge Cognitive Examination for Older Adults with Down's Syndrome (CAMCOG-DS) and the modified Cued Recall Tests (mCRT) are useful for the diagnosis of prodromal AD and AD dementia in adults with DS at a single-point assessment when stratifying by the level of ID.[19] Consequently, some other authors claimed the need for defining clinical cut-offs for screening AD in DS,[16,20] and proposed specific cutoff points.[21-23] However, the proposed cutoffs were derived from ROC analyses (as opposed to the population norms) maximizing the differentiation between groups and differ significantly between studies. On the other hand, a recent longitudinal study found large variance in the intra-individual longitudinal cognitive scores, as well as practice and floor effects in the neuropsychological tests, which may obscure to a certain extent the longitudinal AD-related cognitive decline.[8]

In short, recent studies have shown the possibility to diagnose symptomatic AD using cross-sectional neuropsychological assessments.[21-23] In this study we provide evidence of the diagnostic accuracy of cross-sectional and longitudinal cognitive change for prodromal AD and AD dementia in a population-based cohort of adults with DS to challenge two commonly held assumptions: (i) the superiority of longitudinal assessments over cross-sectional evaluations and (ii) the impossibility to derive population norms to diagnose objective cognitive impairment in adults with DS. The results of this paper may lead to a change of paradigm in the use of neuropsychological tests for the diagnosis of prodromal AD and AD dementia in individuals with DS.

METHODS

Study Design, Setting and Participants: This is a single-center, prospective, longitudinal cohort study of adults with DS recruited from November 2012 to October 2022 at the Alzheimer Down Unit of the Catalan Down Syndrome Foundation and Hospital of Sant Pau, in Barcelona, Spain. We included adults of both sexes, over 18 years of age from a population-based health plan designed to screen for symptomatic AD in adults with DS in Catalonia. This health plan includes structured semiannual or annual neurological and neuropsychological assessments by experienced clinicians. We included individuals with complete cognitive assessment including the Cambridge Cognitive Examination for Older Adults with Down's syndrome (CAMCOG-DS) and the modified Cued Recall Test (mCRT), with mild or moderate levels of ID. We excluded individuals with severe and profound ID as we have previously shown that most are not able to complete the neuropsychological assessments.[8,21] Individuals with medical, pharmacological, or psychiatric conditions interfering with cognition or activities of daily living were also excluded.

The study was approved by the Sant Pau Research Ethics Committees, following the standards for medical research in humans recommended by the Declaration of Helsinki. All participants or their legally authorized representative gave written informed consent before enrollment. Confidentiality was guaranteed in accordance with current Spanish legislation (LOPD 3/2018).

Study Outcomes: The study procedures included a complete medical examination with the participant and main caregiver, as well as a complete neuropsychological assessment, including the CAMCOG-DS Spanish version[110] and the mCRT,[25] whenever possible.[8,21,26] ID

was categorized as mild, moderate, severe or profound according to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition, and based on caregivers' reports of the individuals' best-ever level of functioning, taking into consideration also the Intelligence quotient obtained at baseline with the Kaufman Brief Intelligence Test Spanish version.[27]

The CAMCOG-DS is a cognitive battery comprising subscales for 7 cognitive domains: orientation, language, memory, attention, praxis, abstract thinking, and perception. The maximum score is 109 and higher scores indicate better cognition.[24] The mCRT is an adapted test to assess free and cued episodic memory in people with ID and it gives two memory measures, free immediate recall (FIR) score (maximum score of 36), and the Total Immediate Recall (TIR), which is the sum of FIR and cued recall; higher scores also indicate better cognition.[25,28] In addition to the raw values at each time-point, we calculated the longitudinal cognitive change (ΔCAMCOG-DS and ΔCRT subscores) with the following formula: follow-up cognitive score – baseline cognitive score.

Participants were clinically classified into 4 groups: i) asymptomatic (aDS): no clinical or neuropsychological suspicion of AD (absence of cognitive impairment beyond the ID or functional decline compared to previous functioning); ii) prodromal AD (pDS): suspicion of AD, but symptoms did not fulfill criteria for dementia (evidence of cognitive impairment without any additional functional impairment); iii) AD dementia (dDS): full blown AD dementia (evidence of cognitive impairment that interfered with everyday activities); IV) uncertain or non-degenerative neurocognitive disorder (ie, when there were medical, pharmacological, or psychiatric condition interfering with cognition or daily living activities, but no suspicion of neurodegenerative origin). We excluded all the visits with an uncertain diagnosis. Importantly, this clinical classification is performed independently by the neurologist and the neuropsychologist before a consensus meeting between both professionals to determine the final diagnosis. As in our previous studies, for the current analysis we used the neurologist diagnosis to avoid circularity in our data.[21]

To better reflect the diagnostic performance of the neuropsychological tests at different time-points, we included all data points from baseline for each diagnostic category and, for prodromal AD and AD dementia we also included the data points of those patients who progressed along the AD continuum after the change in diagnostic category as a new baseline.

Statistical analysis: To assess the descriptive statistics for the baseline data, we performed ANOVA tests for numeric variables and chi-squared tests of independence for categorical variables. Analyses were performed in STATA 15 software.

We performed receiver operating characteristic curve (ROC) analyses on the cross-sectional cognitive scores and the longitudinal cognitive change to compare their Areas Under the Curve (AUC). We first described the AUC at baseline and at the different time-points. We next analyzed the longitudinal cognitive changes at the different time-points by diagnostic group to assess the variability. Then, we performed a test of equality of ROC areas to compare the diagnostic performance of cross-sectional with the annual cognitive change and the combination of both measures. Third, we compared the AUCs and cut-offs derived from the ROC analyses when selecting the symptomatic subjects at baseline and at the different follow-ups to assess the stability of these measures with disease progression (as an estimate of the robustness of the cut-offs with different sample compositions). As an alternative, we finally propose cut-offs derived from the normative data in young asymptomatic adults with DS (age≤35 years) by defining the scores at percentile ranks of 1st, 5th, and 10th in the mild and moderate ID separately, as previously done.[119]

Table 1. Demographic and cognitive data by clinical diagnosis at baseline.

Whole sample, n=589	Asymptomatic, n=440	Prodromal, n=63	Dementia= 86
Sex (n, % female)	207 (47.05%)	36 (57.14%)	42 (48.84%)
Age mean (SD)	37.05 (9.59)	51.55 (4.78)	52.77 (5.55)
Follow-up, months mean (SD)	51.8 (26.8)	22.02 (14.9)	25.3 (18.7)
ID n (%)			
mild	152 (34.55%)	12 (19.05%)	12 (13.95%)
moderate	288 (65.45%)	51 (80.9%)	74 (86.05%)
CAMCOG-DS (n, mean (sd))			
mild – Baseline,	149, 87.29 (7.23)	12, 78.58 (6.86)	11, 59.27 (19.66)
1-year ΔCog	95, 1.55 (4.89)	17, -3.18 (6.40)	24, -5.67 (7.12)
moderate - Baseline	277, 67.75 (13.43)	46, 58.48 (10.12)	71, 44.42 (13.74)
1-year ΔCog	154, 1.42 (6.10)	72, -4.94 (9.56)	44, -1.5 (6.05)
mCRT TIR (n, mean (sd))			
mild - Baseline	141, 35.37 (1.44)	10, 27.5 (7.56)	11, 17.09 (9.80)
1-year ΔCog	86, 0.17 (1.25)	15, -1.27 (3.61)	19, -1.05 (5.87)
moderate - Baseline	242, 33.66 (3.84)	49, 25.16 (8.48)	40, 18.43 (8.71)
1-year ΔCog	128, 0.64 (2.41)	38, -1.13 (6.38)	33, -1.58 (6.65)
mCRT FIR (n, mean (sd))			
mild - Baseline	141, 20.85 (4.46)	10, 13.3 (3.97)	11, 7.82 (5.72)
1-year ΔCog	86, 0.98 (4.02)	15, -0.87 (4.60)	18, -2.39 (3.05)
moderate	242, 16.96 (5.69)	49, 10.08 (5.31)	40, 7.85 (4.58)
1-year ΔCog	128, 0.56 (4.60)	38, -0.68 (4.89)	33, -1.94 (4.23)

Abbreviations: AD, Alzheimer disease; CAMCOG-DS, Cambridge Cognitive Examination for Older AdultsWith Down Syndrome; mCRT TIR, modified Cued RecallTest Total Immediate Recall; mCRT FIR, modified Cued RecallTest Free Immediate Recall; sd, standard deviation; ΔCog, longitudinal cognitive change.

RESULTS

Study population: We included a total of 589 adults with DS from November 2012 to October 2022 with a total of 2119 cognitive evaluations. The mean (SD) time of follow-up was 23.7 (25.1) months, the whole sample mean (SD) age at baseline was 40.9 years (10.9) and 48.4% were female. Table 1 displays the demographic and cognitive data by clinical group: 440 individuals (74.7%) had aDS, 63 (10.7%) pDS, and 86 (14.6%) dDS. As expected, aDS were younger and had higher cognitive scores than pDS and dDS participants (p-value: <0.001 for all comparisons). There were no significant differences in sex distribution across groups. aDS had a longer follow-up period than pDS and dDS (p-value: <0.000). Table 1 shows median scores and standard deviation for baseline cognitive scores and for the longitudinal change at different time-points. As expected, all cognitive scores were higher in aDS than pDS and dDS (p-value: <0.001 for all comparisons). Supplementary table 1 shows the longitudinal cognitive decline at each time-point of follow-up.

Diagnostic performance of the baseline vs. longitudinal cognitive assessments: We conducted ROC analyses on the cross-sectional cognitive scores (csCog) and the longitudinal cognitive change (ΔCog) at baseline and at the 4 different time-points of follow-up to describe the diagnostic performance of the CAMCOG-DS and the mCRT, stratified by ID.

Figure 1. AUCs of the three cognitive measures at baseline and at 4 different time-points in prodromal AD and in AD dementia stratified by level of ID. thin lines represent the AUCs along the different years of follow-up, thick lines represent the AUC of baseline scores, blue color corresponds to pDS groups and red color to dDS group

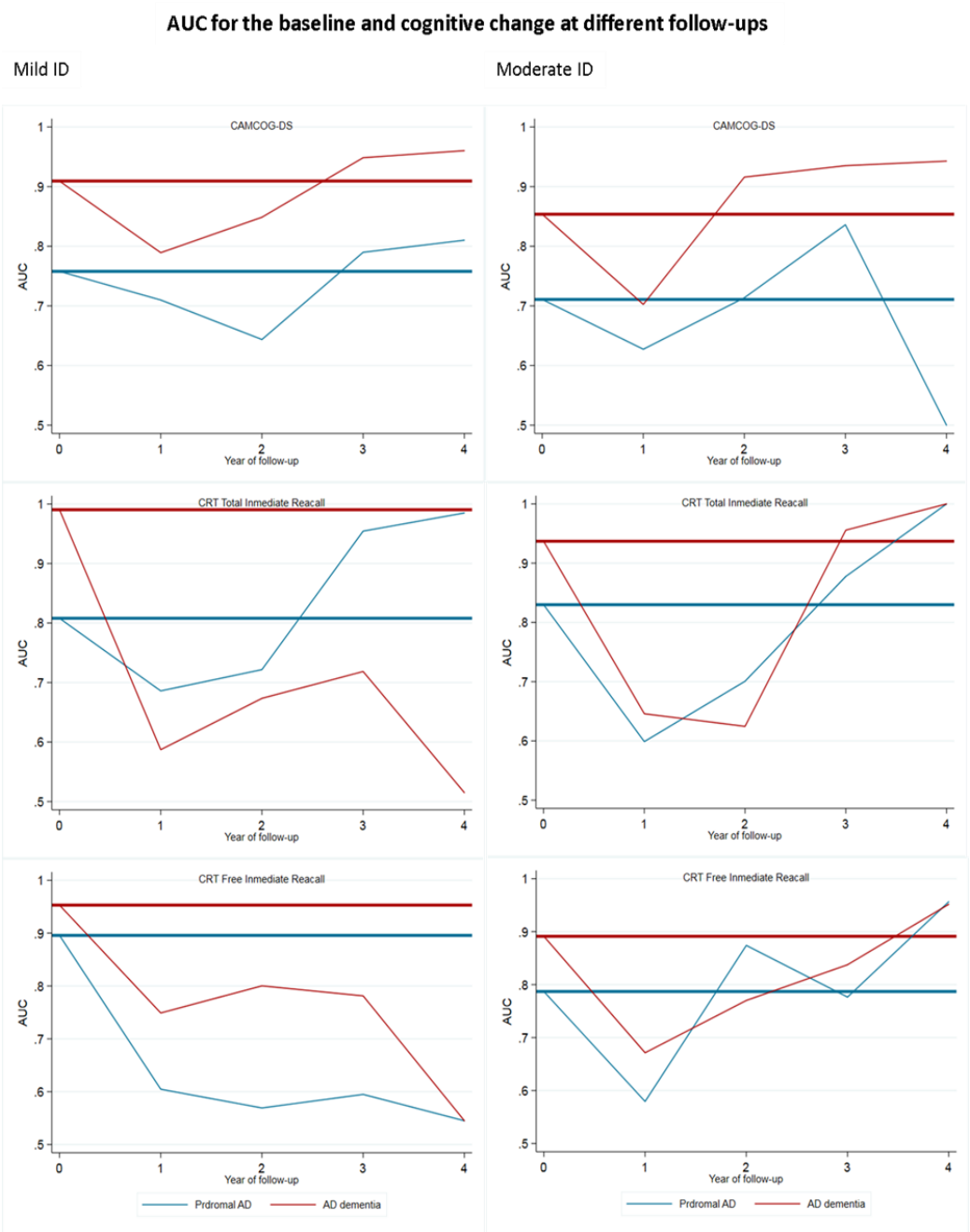


Figure 1 and supplementary figure 1 show the AUCs of the three cognitive measures at baseline and at 4 different time-points. Baseline mCRT TIR and FIR scores showed good to excellent diagnostic performances to distinguish between aDS with pDS (TIR AUC were 0,808 and 0,8299 for mild and moderate levels of ID, respectively; and FIR AUCs were 0,8957 and 0,787 for mild and moderate levels of ID, respectively) and dDS (TIR AUCs were 0,9904 and 0,937 for mild and moderate levels of ID, respectively; and FIR AUCs were 0, 0,953 and 0, 0,9117 for mild and moderate levels of ID, respectively). Baseline CAMCOG-DS showed the lowest diagnostic performance to detect pDS in the whole sample, as well as when analyzing mild and moderate ID separately (AUCs 0.7137, 0.7579 and 0.7108, respectively).

The 1-year longitudinal cognitive change of the three cognitive measures showed a worse diagnostic performance to diagnose prodromal AD and AD dementia than baseline measures (all AUCs<=0.72, except in the CAMCOG-DS for the AD dementia diagnosis in the mild ID group that had an AUC of 0.8). The AUC for the longitudinal cognitive change progressively increased with longer follow-ups surpassing the baseline diagnostic performance after 2 or 3 years, except for the FIR measure, which never achieved the baseline AUC. Of note, by the fifth year of follow-up most symptomatic AD patients have evolved to a more severe cognitive decline and, thus, the neuropsychological assessment was not feasible due to floor effects. Supplementary table 2 shows the AUCs of each cognitive measure at baseline and the different years of follow-up.

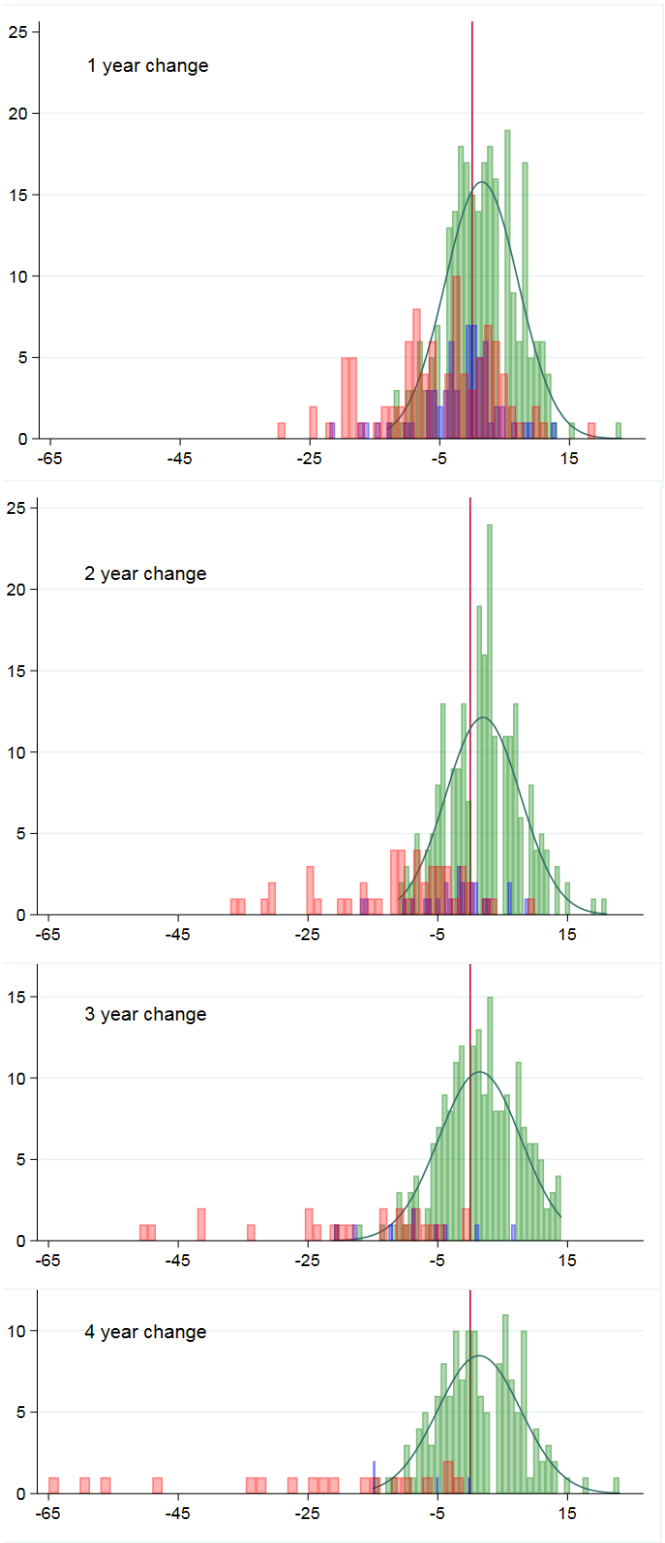
To assess whether the lower diagnostic performance of the longitudinal assessments with respect the baseline evaluation was due to the variability in the intra-individual cognitive trajectories we performed several analyses. First, supplementary figure 2 shows the spaghetti plots for the longitudinal trajectories of the cognitive scores, and illustrates that individual cognitive performance vary greatly in these measures. Second, figure 2 shows the longitudinal cognitive change at each annual visit in the different clinical groups. As shown in table 2, 56.2% of aDS, but also 32.8% of pDS and 30.2% of dDS patients improved the cognitive performance in the CAMCOG-DS at the 1-year evaluation. However, with longer follow-up periods, the AD-related cognitive declines increase the signal to noise ratio,

Table 2. Table 2 shows the percentage of cognitive change with respect to baseline at each different year of follow-up by clinical group.

		YEAR1	YEAR2	YEAR3	YEAR4	YEAR5
aDS	Mean (SD)	1.47 (5.66)	1.98 (5.76)	1.48 (6.32)	1.39 (6.40)	1.27 (6.47)
	No change	6,02%	3,20%	6,38%	6,99%	10,75%
	Improve %	56,22%	63,47%	56,38%	53,85%	52,69%
	Worsen %	37,75%	33,33%	37,23%	39,16%	36,56%
pDS	Mean (SD)	-1.97 (6.14)	-2.54 (6.27)	-7.78 (8.79)	-8.75 (7.5)	-10 (7.39)
	No change	11,48%	8,33%	0,00%	25,00%	0,00%
	Improve %	32,79%	29,17%	22,22%	0,00%	0,00%
	Worsen %	55,74%	62,50%	77,78%	75,00%	100,00%
dDS	Mean (SD)	-5.16 (8.98)	-11.29 (10.58)	-19.30 (14.94)	-25.44 (20.09)	-47.5 (40.31)
	No change	3,13%	4,17%	4,35%	0,00%	0,00%
	Improve %	30,21%	6,25%	0,00%	0,00%	0,00%
	Worsen %	66,67%	89,58%	95,65%	100,00%	100,00%

Figure 2. Longitudinal cognitive change at each annual visit in the different clinical groups (green color corresponds to aDS group, blue color corresponds to pDS and red color corresponds to dDS). Vertical red line shows the 0 annual change, bars over this line represents all those individuals which improve their cognitive performance, and bars below the red line represents those individuals which cognitive performance worsen.

Longitudinal cognitive change at each annual



	Population norms cut-offs								ROC analysis	
	1%		5%		10%		15%		Baseline	
	pDS	dDS	pDS	dDS	pDS	dDS	pDS	dDS	pDS	dDS
CAMCOG-DS										
Mild	**72** 5.56%/99.6%	**72** 70.7%/99.6%	**78** 25.9%/97.2%	**78** 88.9%/ 97.2%	**81** 42.6%/91.2%	**81** 96.0%/91.2%	**82** 48.1%/88.5%	**82** 96.0%/88.5%	**87** 92%/55%	**81** 85%/83%
Moderate	**41** 5.84%/98.8%	**41** 35.2%/98.8%	**50** 25.3%/93.7%	**50** 58.0%/93.7%	**55** 36.4%/87.3%	**55** 71.2%/87.3%	**59** 52.6% /80.6%	**59** 81.6% /80.6%	**65** 73%/63%	**56** 73%/82%
mCRT TIR										
Mild	**32** 44.0%/ 99.2%	**32** 93.9%/ 99.2%	**35** 78.0%/92.4%	**35** 100.0%/92.4%	**35** 78.0%/92.4%	**35** 100.0%/92.4%	**35** 78.0%/92.4%	**35** 100.0%/92.4%	**35** 67%/89%	**34** 100%/92%
Moderate	**29** 59.0%/ 97.8%	**29** 86.2%/ 97.8%	**32** 76.3%/ 93.5%	**32** 93.1%/ 93.5%	**33** 79.1%/ 90.3%	**33** 94.6%/ 90.3%	**34** 83.5%/83.6%	**34** 96.9%/83.6%	**33** 73%/81%	**30** 86%/89%
mCRT FIR										
Mild	**11** 24.0%/99.0%	**11** 67.7%/ 99.0%	**16** 54.0%/ 92.2%	**16** 87.7%/ 92.2%	**18** 76.0% /84.6%	**18** 98.5%/84.6%	**20** 88.0%/ 75.5%	**20** 100.0%/75.5%	**19** 100%/74%	**17** 92%/85%
Moderate	**3** 6.47%/98.9%	**3** 13.8%/98.9%	**11** 52.5%/ 94.5%	**11** 77.7%/ 94.5%	**12** 61.9%/ 92.7%	**12** 81.5%/ 92.7%	**15** 79.1%/ 82.1%	**15** 92.3%/ 82.1%	**15** 73%/73%	**12** 79%/87%

enabling more robust diagnoses.

Finally, we explored the diagnostic performance of combining the cross-sectional and the 1-year cognitive change. The combination of these measures improved the diagnostic performance for prodromal AD, but not for AD dementia (supplementary figure 3).

Sensitivity of ROC-derived cut-offs to sample composition: To assess the sensitivity of ROC-derived cut-offs to sample composition (i.e. the severity of the symptomatic AD cases included), we performed ROC analyses to derive the AUCs and the cut-offs when selecting the scores at baseline, and when selecting the scores at the different years of follow-up (as a proxy of disease severity). As shown in Figure 3, cut-offs and AUCs changed markedly depending on the sample composition. The AUCs increased and the cutoff decreased when more advanced cases were included due to the progression of cognitive decline in the symptomatic participants.

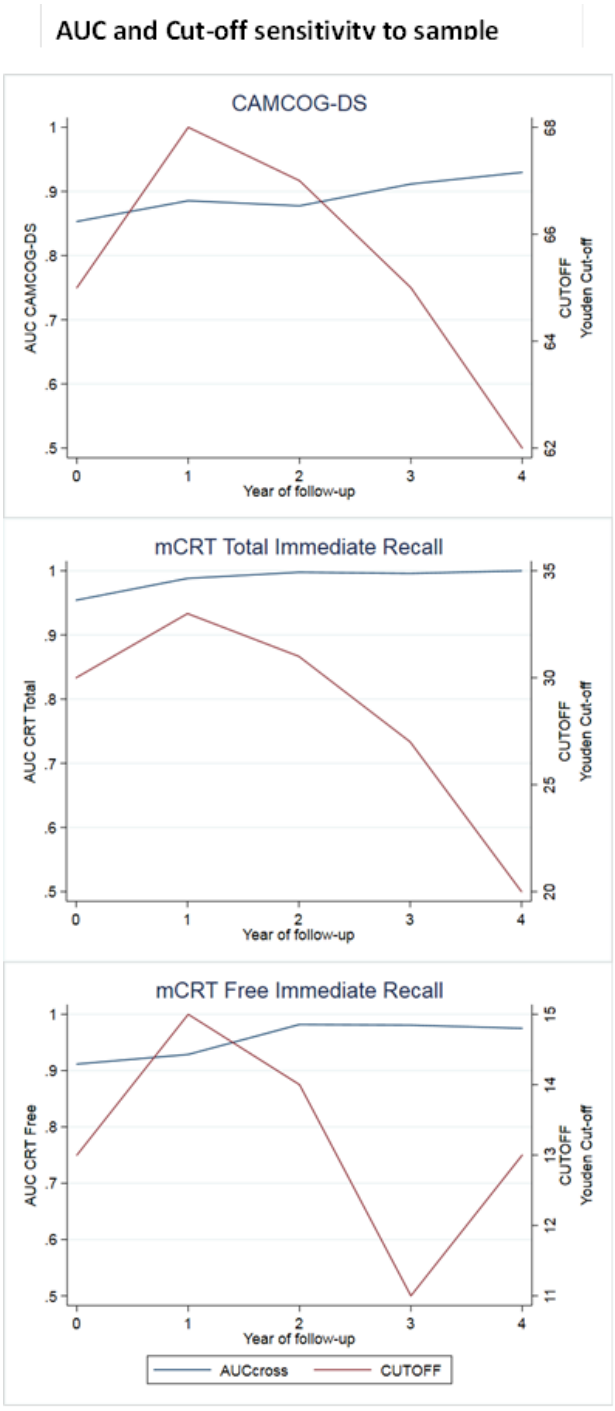
Normative data for the CAMCOG-DS and mCRT in asymptomatic DS individuals: Finally, after showing the great influence of sample composition on the cut-offs derived from the ROC analyses, we explored the sensitivity and specificity of the cut-offs derived from population norms. To exclude cognitive impairment due to preclinical AD, we derived normative data in the asymptomatic younger subjects (≤35 years: n=174 for the CAMCOG-DS and 160 for the mCRT), in mild and moderate ID groups separately. Table 3 shows the scores corresponding to the 1st, 5th, 10th ,15th percentiles and the sensitivity and specificity of each cutoff for prodromal AD and AD dementia, as well as the cut-offs derived from the ROC analysis of baseline score, for prodromal AD and AD dementia separately and stratified by ID.

Cutoffs derived from percentile 5% were 78 and 50 for the CAMCOG-DS in mild and moderate ID groups, respectively. These cut-offs showed high specificity, but low sensitivity, except for the mild ID group, were sensitivity was 89%. However, the 5% percentile derived cutoffs for the mCRT TIR showed higher sensitivities and specificities in mild and moderate ID, which were 35 in the mild ID group (sensitivity/specificity of 78.0%/92.4% for prodromal AD and 100.0%/92.4% for dementia), and 32 in the moderate ID group (sensitivity/specificity of 76.3%/ 93.5% for prodromal AD and 93.1%/ 93.5% for dementia). Cut-offs for the mCRT FIR of 16 for mild ID and 11 for moderate ID showed acceptable to excellent sensitivity and specificity for AD dementia in both groups, but lower sensitivity for prodromal AD.

DISCUSSION

To the best of our knowledge, this study compares for the first time the diagnostic performance of cross-sectional and longitudinal neuropsychological assessments to detect symptomatic AD in adults with DS. It shows that not only cross-sectional neuropsychological assessments accurately diagnose prodromal AD and AD dementia in adults with DS, but also that the cross-sectional

Figure 3. Sensitivity of ROC-derived cut-offs and AUCs to sample composition along time in the symptomatic AD DS patients. Blue color corresponds to AUC and red color to ROC-derived cutoffs.



evaluation outperforms the 1-year longitudinal cognitive change, challenging current diagnostic recommendations and emphasis on the intra-individual change for diagnosis. We also show the instability of the cut-offs including symptomatic individuals with different disease severity and assess the use of population norms for individuals with DS like it is customary in the general population.

Contrary to current diagnostic guidelines,[18] the longitudinal cognitive change at one year was less useful than single-point evaluations for the diagnosis of prodromal AD and AD dementia. This controversial result might be partly explained by the intra-individual cognitive variability observed in DS. Of note, the 2-year longitudinal assessment yielded similar results to the baseline evaluation due to the higher signal to noise ratio (or power of the 2/3-year change over the within-individual longitudinal variability), especially in the CAMCOG-DS. Other factors that might contribute to the lower performance of the one-year longitudinal assessments include the practice effects seen in repeated measurements, and the ceiling and floor effects observed in the mCRT at different stages of the disease, which also reduced the power of the longitudinal assessments.[8] This variability with repeated measurements is not specific of DS, but also found in the general population. Day-to-day variability in mood, fatigue and stress can significantly impact participants' attention and thus influence in cognitive performance.[29-31] In fact, longitudinal assessments do not offer substantial benefit over cross-sectional assessment in detecting preclinical AD or incident MCI in the general population either.[32,33]

We also showed the inconsistency of cut-offs derived from the ROC analyses due to an intrinsic sample composition bias. ROC analyses have been used in previous studies in DS, including the validation of the Spanish version of CAMCOG,[24] and the original and the French validation of the CRT.[22,23,25] Importantly, the proposed cutoffs were not congruent across studies. The original cutoff found for the mCRT TIR to detect symptomatic AD was 23 with high sensitivity and specificity (0.939/0.947, respectively),[25] similar to the French validation (cut-off 22, sensitivity and specificity of 0.92 and 1.00, respectively).[22] However, other works reported higher cut-offs, for example a recent work found scores ≥33 in the mCRT TIR had an excellent sensitivity with limited specificity for the detection of prodromal AD in DS, and scores ≤20 for dementia.[23] Only one previous study proposed different cut-offs for individuals

with mild and moderate ID separately, which were also higher than the first two studies mentioned (for mild ID the cutoff (sensitivity/specificity) proposed was 35 (66.7%/73.3%) to detect prodromal AD and 29 (100%/100%) for dementia, and in the moderate ID group 33 (78.3%/79.2%) for prodromal AD and 28 (92.3%/94.4%) for dementia).[21] In our present study, we show how ROC analyses are very sensitive to sample composition (i.e. samples of symptomatic individuals with different disease severity) and might explain some of the reported discrepancies in the reported cutoffs.

In contrast, cutoffs derived from population norms are more stable than those derived from the ROC analyses, and thus are preferred and generally used in the general population. To avoid the cognitive decline in preclinical AD, we selected young asymptomatic individuals with DS (aged ≤ 35 years) with mild and moderate levels of ID to derive the population norms. Similar to the general population, cut-offs derived from the 5% percentile in the mCRT TIR showed high sensitivity and specificity for the diagnosis of prodromal AD (cut-offs of 35 and 32 for mild and moderate respectively). However, the CAMCOG-DS and the mCRT FIR had lower sensitivity with high specificity, which improved in the derived cutoffs from the 10^{th} and 15^{th} percentiles. In line with our previous work,[8] we propose the mCRT as a test for the early diagnose of symptomatic AD in DS and the CAMCOG-DS for monitoring AD-related cognitive decline similar to ADAS-cog in the general population. Despite these limitations, from a neuropsychological perspective, this result brings together the cognitive assessment of AD-related changes in SD to that in the general population and might enable more robust cut-offs in multicenter studies.

The main strengths of this study are the large sample size and the fact that it comes from a large population-based cohort of adults with DS. This enabled us to compare the cross-sectional vs. longitudinal cognitive data and propose population-based norms for mild and moderate ID derived from young aDS. This study also has some limitations. First, it is a single-center study, and thus needs to be replicated in other cohorts and populations to confirm the generalizability of our results. Second, to avoid circularity, we used the neurologist's diagnosis, blinded to neuropsychological assessment, and this could have led to a misidentification of prodromal AD cases as asymptomatic or to the inclusion in the asymptomatic group of subjects with medical, pharmacological, or psychiatric conditions that could have effects on cognition not detected by the neurologist. Of note this might have underestimated the diagnostic performance of the tests. Future works in other cohorts must confirm the robustness of the population norms, which would increase the diagnostic confidence and greatly enhance multinational collaborations.

In brief, baseline neuropsychological evaluation showed a higher diagnostic performance than the annual longitudinal cognitive change. If these population-based normative values prove robust in multicenter studies, they can lead to a change in clinical practice akin to that established in the general population.

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Diagnostic and prognostic performance and longitudinal changes in plasma neurofilament light chain concentrations in adults with Down syndrome: a cohort study

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SUMMARY

Background: Adults with Down syndrome are at an ultra-high risk of Alzheimer's disease, but diagnosis of Alzheimer's disease in this population is challenging. We aimed to validate the clinical utility of plasma neurofilament light chain (NfL) for the diagnosis of symptomatic Alzheimer's disease in Down syndrome, assess its prognostic value, and establish longitudinal changes in adults with Down syndrome.

Methods: We did a multicentre cohort study, including adults with Down syndrome (≥18 years), recruited from six hospitals and university medical centres in France, Germany, Spain, the UK, and the USA, who had been assessed, followed up, and provided at least two plasma samples. Participants were classified by local clinicians, who were masked to biomarker data, as asymptomatic (ie, no clinical suspicion of Alzheimer's disease), prodromal Alzheimer's disease, or Alzheimer's disease dementia. We classified individuals who progressed along the Alzheimer's disease continuum during follow-up as progressors. Plasma samples were analysed retrospectively; NfL concentrations were measured centrally using commercial kits for biomarker detection. We used ANOVA to evaluate differences in baseline NfL concentrations, Cox regression to study their prognostic value, and linear mixed models to estimate longitudinal changes. To account for potential confounders, we included age, sex, and intellectual disability as covariates in the analyses.

Findings: Between Aug 2, 2010, and July 16, 2019, we analysed 608 samples from 236 people with Down syndrome: 165 (70%) were asymptomatic, 32 (14%) had prodromal Alzheimer's disease, and 29 (12%) had Alzheimer's disease dementia; ten [4%] participants were excluded because their classification was uncertain. Mean follow-up was 3·6 years (SD 1·6, range 0·6–9·2). Baseline plasma NfL concentrations showed an area under the receiver operating characteristic curve of 0·83 (95% CI 0·76–0·91) in the prodromal group and 0·94 (0·90–0·97) in the dementia group for differentiating from participants who were asymptomatic. An increase of 1 pg/mL in baseline NfL concentrations was associated with a 1·04-fold risk of clinical progression (95% CI 1·01–1·07; p=0·0034). Plasma NfL concentrations showed an annual increase of 3·0% (95% CI 0·4–5·8) per year in the asymptomatic non-progressors group, 11·5% (4·9–18·5) per year in the asymptomatic progressors group, and 16·0% (8·4–24·0) per year in the prodromal Alzheimer's disease progressors group. In participants with Alzheimer's disease dementia, NfL concentrations increased by a mean of 24·3% (15·3–34·1). Interpretation Plasma NfL concentrations have excellent diagnostic and prognostic performance for symptomatic Alzheimer's disease in Down syndrome. The longitudinal trajectory of plasma NfL supports its use as a theragnostic marker in clinical trials.

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INTRODUCTION

Down syndrome (trisomy 21) is the most common genetic cause of intellectual disability, affecting 5·8 million people worldwide.[1] Due to the extra copy of the amyloid precursor protein gene caused by trisomy of chromosome 21, nearly all adults with Down syndrome have Alzheimer's disease neuropathology in their 40s, and have an ultra-high (>95%) lifetime risk of developing symptoms of Alzheimer's disease.[2–4] The diagnosis of symptomatic Alzheimer's disease in Down syndrome is difficult, mainly because of the variability in cognitive

performance, due in part to level of intellectual disability, and the absence of validated standardized assessment tools specifically designed for this population. However, core CSF Alzheimer's disease biomarkers (eg, amyloid β1–42, total tau, and tau phosphorylated at threonine 181 [p-tau181]) have proven to be useful in the diagnosis of prodromal (ie, cognitive impairment without substantial impairment in social or occupational functioning) Alzheimer's disease and Alzheimer's disease dementia (ie, substantial cognitive impairment that leads to a loss of independence in activities of daily living) in the general population[5] and in Down syndrome.[6] A diagnostic blood-based biomarker would therefore have clear advantages, particularly for people with Down syndrome for whom acquiring CSF samples can be a challenge. Neurofilament light chain (NfL) is a scaffolding cytoskeletal protein of myelinated subcortical axons that can be reliably measured in plasma through single molecule array (Simoa).[7] NfL is a nonspecific biomarker of axonal damage.[8] In the context of Down syndrome, NfL might also be associated with a diagnosis of symptomatic Alzheimer's disease because alternative diagnoses affecting NfL concentrations are exceedingly rare in early-onset disease.[6] NfL is a treatment-sensitive biomarker in some neurodegenerative diseases.[9,10] NfL concentrations can predict disease progression and brain neurodegeneration in preclinical sporadic and autosomal dominant Alzheimer's disease.[11–14] In Down syndrome, plasma NfL concentrations correlate in cross-sectional studies with CSF concentrations of total and p-tau181, and also with cognitive performance.[6,15,16] High NfL concentrations also predict worse adaptive behavior scores at 1 year.[17] However, the prognostic value along the Alzheimer's disease continuum or the longitudinal changes in plasma NfL concentrations have not been assessed in Down syndrome. Leveraging a large cohort of adults with Down syndrome, this multicentre collaborative effort aimed to validate the diagnostic performance of plasma NfL concentrations for symptomatic Alzheimer's disease in Down syndrome; assess the prognostic performance of plasma NfL concentrations; and establish the longitudinal trajectory of plasma NfL concentrations along the Alzheimer's disease continuum in Down syndrome. This information is essential to improve diagnostic accuracy for Alzheimer's disease in Down syndrome and to implement plasma NfL as an outcome measure in Alzheimer's disease clinical trials in Down syndrome.

METHODS

Study design and participants: We included adults with Down syndrome (≥18 years) who had been followed up and evaluated in one of six different centres: Hospital de la Santa Creu i Sant Pau (Barcelona, Spain), University of Kentucky (Lexington, KY, USA), Institute Jérôme Lejeune (Paris, France), King's College London (London, UK), University of Cambridge (Cambridge, UK), and Ludwig-Maximilians-University of Munich (Munich, Germany). Participants with Down syndrome had to have two or more plasma samples available and information of their cognitive status for those time-points. Following the recommendations of the National Task Group on Intellectual Disabilities and Dementia Practices Consensus Recommendations for the Evaluation and Management of Dementia in Adults With Intellectual Disabilities,[18] clinical dementia status was determined individually for each participant in a Consensus Case Conference.[4,19,20] These discussions included at least two clinicians with longstanding expertise in evaluating dementia in the Down syndrome population, and included the review of the medical and psychiatric history and findings from the neurological exam, interviews with carers or family members, and the participant's performance in the neuropsychological evaluation, taking into consideration the participants' baseline intelligence quotient, medical and psychiatric conditions, and any major life events.[19] Clinicians were masked to biomarker data.

The participants with Down syndrome were classified into the following groups:

asymptomatic (for those with no clinical or neuropsychological suspicion of Alzheimer's disease); prodromal Alzheimer's disease (for those for whom there was a suspicion of Alzheimer's disease, but symptoms did not fulfil criteria for dementia); or Alzheimer's disease dementia (for those fulfilling criteria for dementia). Functional status to differentiate prodromal Alzheimer's disease and Alzheimer's disease dementia diagnoses was assessed on the basis of medical history, and with the support of validated questionnaires (appendix pp 2–3) to differentiate decline due to cognitive impairment from pre-existing intellectual disability, placing a particular emphasis on establishing change from the individual's best level of functioning.[4,19,20] At each timepoint during follow-up, clinical status was established for every participant by their local clinician (ie, asymptomatic, prodromal, or dementia).Then, progression was defined as a change in the clinical status label of the participants during the follow-up. Participants were classified as progressors if their clinical status label changed from asymptomatic to prodromal or dementia, or from prodromal to dementia) or non-progressors if their clinical status label remained the same. Participants were followed-up routinely following each centre's protocols, which included assessments every 6 months to1 year.

Those participants who had clinically significant medical, pharmacological, or psychiatric conditions considered likely to interfere in cognition or in daily functional tasks were classified as uncertain and excluded from the study. Clinicians who classified the participants were masked to biomarker results, and specifically to NfL concentrations. For prognostic evaluation, participants who were asymptomatic and prodromal were subsequently classified as progressors when there was clinical progression along the Alzheimer's disease continuum or death due to Alzheimer's disease. Those participants who remained in the same diagnostic category at the end of follow-up were classified as non-progressors.

All participants or their legal guardians gave written consent or assent, and the local ethics committee of each centre approved all procedures included in this study.

Procedures: Level of intellectual disability was categorised as mild, moderate, severe, or profound according to the Diagnostic and Statistical Manual of Mental Disorders, fifth edition, based on caregivers' reports of the individuals' best-ever level of functioning. Due to the low number of participants with severe and profound intellectual disability, these two categories were merged for all analyses.
Cognitive assessment included a neurological and neuropsychological examination covering several cognitive domains. Details for cognitive tests at each participating centre are detailed in the appendix (pp 2–3).

After blood collection, all samples were transferred to each local laboratory where they were centrifuged, aliquoted, and frozen at –80°C, following international recommendations. Apolipoprotein E (APOE) genotype was determined at each centre. Plasma samples were shipped in dry ice to the laboratory in Hospital de la Santa Creu i Sant Pau (Barcelona, Spain) where they were stored at –80°C until analysis.

Concentrations of plasma NfL were centrally measured in Hospital de la Santa Creu i Sant Pau using the ultrasensitive equipment Simoa SR-X (Quanterix, Billerica, MA, USA). All samples were measured in duplicate, and within one round of experiments between Aug 1, and Sept 30, 2019, using commercially available kits (NF-light, Quanterix). The intra-assay coefficient of variation was 3·4% and the inter-assay coefficient of variation was 16·7%. Baseline and longitudinal samples obtained from each participant were measured side by side in the same run to avoid the effect of run-to-run variability. All analyses were done by one technician (LM), who was masked to clinical diagnosis. A subset of samples from this study had been previously analysed with Simoa HD-1 equipment (Montpellier, France). There was a

	By baseline diagnosis				By clinical progression				
	Asymptomatic	Prodromal Alzheimer's disease	Alzheimer's disease dementia	p value	Asymptomatic, non-progressor	Asymptomatic, progressor	Prodromal Alzheimer's disease, non-progressor	Prodromal Alzheimer's disease, progressor	p value
Participant baseline samples	165 (70%)	32 (14%)	29 (12%)	NA	135 (57%)	30 (13%)	8 (3%)	24 (10%)	NA
Longitudinal samples	263 (43%)	43 (7%)	40 (7%)	NA	206 (34%)	57 (9%)	9 (2%)	34 (6%)	NA
Age, years	38·9 (9·7)	50·6 (5·5)	53·3 (5)	<0·0001*	36·7 (8·7)	48·9 (7·5)	46·4 (3·4)	52 (5·4)	<0·0001
Sex	..	..	..	0·98†	..	..	..	..	0·81†
Female	75 (45%)	14 (44%)	13 (45%)	..	62 (46%)	13 (43%)	2 (25%)	12 (50%)	..
Male	90 (55%)	18 (56%)	16 (55%)	..	73 (54%)	17 (57%)	6 (75%)	12 (50%)	..
Participants with intellectual disability	..	..	..	0·31†	..	..	..	..	0·69†
Mild	49 (30%)	12 (38%)	5 (17%)	..	41 (30%)	8 (27%)	3 (38%)	9 (38%)	..
Moderate	89 (54%)	18 (56%)	18 (62%)	..	71 (53%)	18 (60%)	4 (50%)	14 (58%)	..
Severe or profound	26 (16%)	2 (6%)	6 (21%)	..	22 (16%)	4 (13%)	1 (13%)	1 (4%)	..
Follow-up time, years	3·8 (1·6)	3·3 (1·5)	2·9 (1·3)	<0·0011*	3·7 (1·5)	4·4 (2·1)	3·3 (1·9)	3·3 (1·4)	0·051*
Plasma NfL concentrations, pg/mL	11·2 (6·5)	22·9 (12·4)	33 (16·5)	<0·0001*	10·2 (6)	15·8 (6·4)	14 (5·4)	25·8 (12·7)	<0·0001*
Plasma NfL adjusted concentrations, pg/mL‡	0·8 (4·7)	8·1 (11·2)	16·3 (16·6)	<0·0001*	0·8 (4·5)	1·3 (5·4)	1·6 (6·6)	10·3 (11·7)	<0·0001*
Apolipoprotein E-ε4 status§	..	..	..	0·92†	..	..	..	..	0·99†
Positive	27 (21%)	6 (23%)	6 (24%)	..	21 (20%)	6 (22%)	2 (25%)	4 (22%)	..
Negative	103 (79%)	20 (77%)	19 (76%)	..	82 (80%)	21 (78%)	6 (75%)	14 (78%)	..

Data are n (%), n/N (%), or mean (SD). Percentages are calculated from numerators shown in participant baseline samples row. NA=not applicable. NfL=neurofilament light chain. *ANOVA. †χ^2 test. ‡Plasma NfL adjusted concentrations were calculated as the difference between measured concentrations and the predicted concentrations estimated from a linear model in the asymptomatic Down syndrome non-progressors group, in whom age together with sex and intellectual disability were considered. §Denominator is the total participants with available apolipoprotein E status in each group or column.

Table: **Baseline characteristics**

high correlation between both assays (r^2=0·94, appendix p 5).

Statistical analysis: Concentrations of plasma NfL were log-transformed to attain a normal distribution. We used ANOVA to compare baseline ages between groups and the χ^2 test to compare the proportion of sex (male or female), intellectual disability, and APOE-ε4 status across diagnostic categories. The association of plasma NfL concentrations with baseline age, sex, and intellectual disability was assessed by analysis of covariance in the group of asymptomatic non-progressors.

Down syndrome is a genetically determined form of Alzheimer's disease.21 Thus, age is intrinsically and robustly linked to the development of symptomatic Alzheimer's disease.[4] For this reason, age was included as a covariate together with sex and intellectual disability in all analyses throughout this manuscript. However, as this approach could potentially obscure the relationships between these two intrinsically linked variables, we confirmed the analysis following an alternative approach. We calculated W-scores applying a linear model in the asymptomatic non-

progressors including age, sex, and level of intellectual disability. Using this model, W-scoreadjusted plasma NfL concentrations were calculated in the whole sample as the difference between those measured and those predicted. Results based on W-scoreadjusted values and also the analyses based in raw NfL values are available in the appendix (pp 3–4).

We did receiver operating characteristic (ROC) analysis for baseline plasma NfL concentrations to calculate areas under the curve with 95% CIs,[22] and we selected cutoff values that maximised the Youden J index (ie, sensitivity plus specificity minus 1). Clinical progression and its association with baseline plasma NfL concentrations were assessed by modelling Kaplan-Meier curves and multivariable Cox regression analysis. Longitudinal changes in plasma NfL concentrations and their association with clinical progression status were assessed through linear mixed models. The initial model included baseline NfL concentrations, diagnostic category, age, sex, intellectual disability, and time from baseline sample (years) including its interaction with diagnostic category as fixed effects. We included a random intercept for centre and for assay run to account for inter-centre and inter-run variability. Random intercepts and slopes were defined at the participant level to account for repeated measures. Outliers were detected by visual inspection of their influence on the residuals. We used backward selection to choose the final model. The study size resulted from all the participants that fulfilled the inclusion criteria (all the participants with two or more plasma samples and two or more clinical and cognitive assessments). Individuals who were lost to

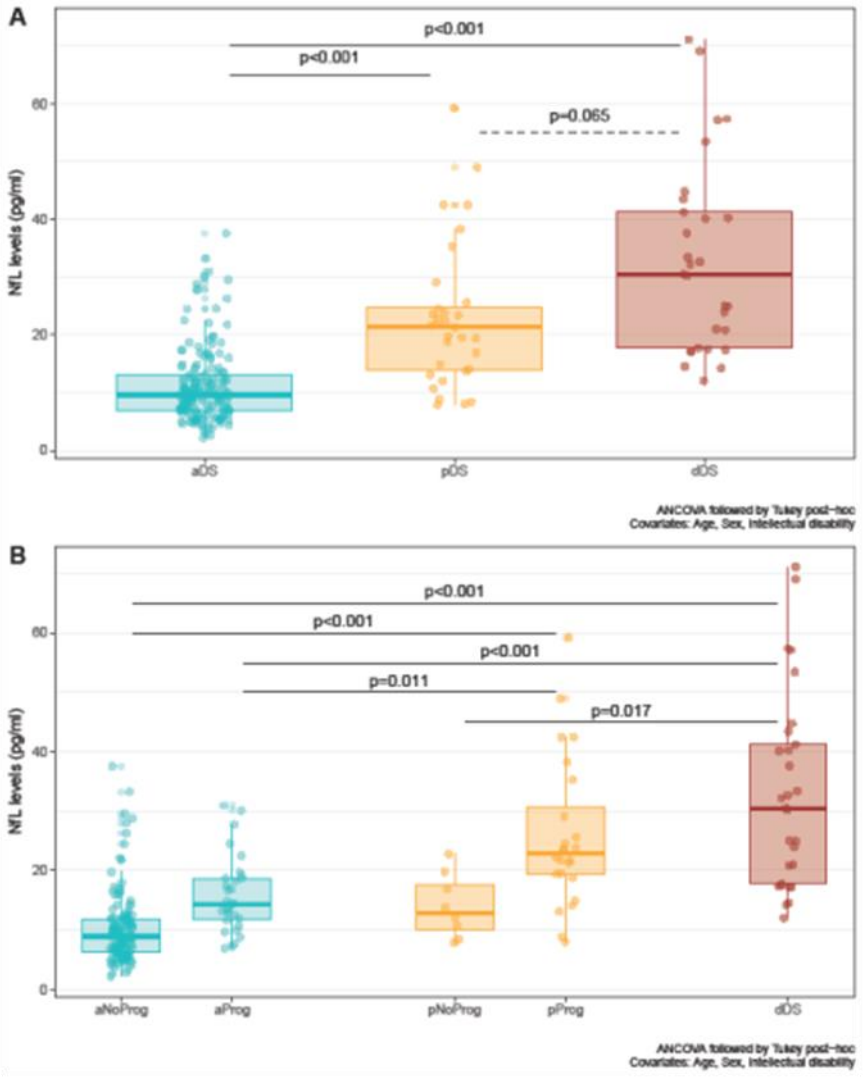

Figure 1: **Baseline plasma NfL concentrations**
Baseline plasma NfL concentrations across baseline categories (A) and across categories considering clinical progression during follow-up (B) in Down syndrome. Age, sex, and intellectual disability were included as covariates in the analysis. Data were analysed using ANCOVA followed by Tukey post-hoc analysis. Only significant associations are shown. NfL=neurofilament light chain.

follow-up before obtaining the second plasma sample or undergoing the second clinical and cognitive assessment were not considered for the study. All the participants included in the study had follow-up information (plasma and clinical or cognitive information).

We used packages car, version 3.0-7, pROC, version 1.16.2, survival, version 3.1-12, survminer, version 0.4.6, nlme, version 3.1-147, multcomp, version 1.4-13, ggplot2, version 3.3.0, and ggpubr version 0.3.0, as implemented in R statistical software, version 3.6.2 for plots and statistical analysis (references are shown in appendix p 19).

Role of the funding source: The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

RESULTS

Between Aug 2, 2010, and July 16, 2019, 608 plasma samples were obtained from 236 participants with Down syndrome. The 236 candidates who had plasma samples available for analysis at baseline and follow-up were examined for eligibility, from which 226 were confirmed eligible and were included in the study (table). The clinical classifications were asymptomatic (n=165 [70%]), prodromal Alzheimer's disease (n=32 [14%]), and Alzheimer's disease dementia (n=29 [12%]). Participants classified as uncertain (n=10 [4%]) were excluded from the study (appendix p 6 for

more details on this group). The table shows demographic, clinical, and biomarker variables across groups by baseline diagnosis and by clinical progression of all participants (n=226) included in the analysis. Participants who were asymptomatic with regard to Alzheimer's disease were significantly younger than those in the prodromal Alzheimer's disease and Alzheimer's disease dementia groups (38·9 years vs 50·6 years vs 53·3 years). Within the asymptomatic group, those who remained stable during follow-up were younger than those who showed clinical progression. Participants were followed up for a mean of 3·6 years (SD 1·6, range 0·6–9·2), although follow-up time was shorter in the Alzheimer's disease dementia group than in participants who were asymptomatic. There were no significant differences in the distribution of sex, intellectual disability, or APOE-ε4 status across groups.

To assess the influence of demographics and level of intellectual disability on NfL concentrations, we assessed the asymptomatic non-progressors group. We found significant associations of age, sex, and intellectual disability with baseline plasma NfL concentrations. An increase of 1 year in baseline age was associated with a 3·8% increase in plasma NfL concentrations (p<0·0001), male participants showed 14·8% lower concentrations of plasma NfL than female participants (p=0·020), and there was weak evidence of a linear association between plasma NfL and level of intellectual disability (β=1·16; p=0·049). To account for these potential confounders, we included age, sex, and intellectual disability as covariates in the analysis. We repeated the analysis with W-score-adjusted concentrations that were calculated as the difference between actual measured concentrations and the predicted concentrations estimated from a linear model in the asymptomatic non-progressors group, in which age together with sex and intellectual disability were considered. The analysis of W-score-adjusted concentrations and that of raw values are shown in the appendix (pp 9–11).

Figure 1 shows baseline plasma NfL concentrations across categories. After adjusting for age, sex, and intellectual disability, concentrations of NfL were 79% higher (p<0·0001) in those with Alzheimer's disease dementia and 40% higher (p=0·0004) in the prodromal group than in the asymptomatic group (figure 1A). The effect was similar when

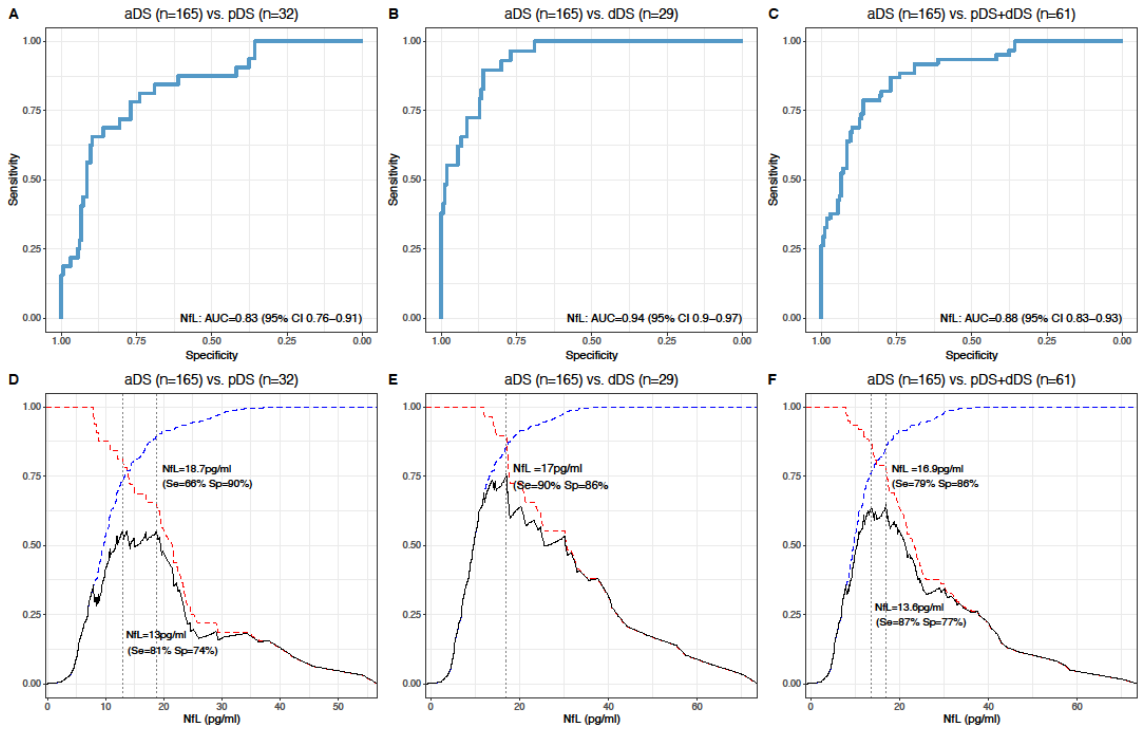

Figure 2: **ROC analysis of baseline plasma NfL concentrations for discrimination of participants who were asymptomatic or symptomatic**
Panels A, B, and C show the ROC curves of NfL to discriminate between clinical categories. Vertical dashed lines in panels D, E, and F indicate cutoff values that yielded an optimal balance between sensitivity and specificity (maximum Youden J index). When two cutoff values yielded the same Youden J indices, both values are indicated (D and F). ROC=receiver operating characteristic. AUC=area under the curve. NfL=neurofilament light chain.

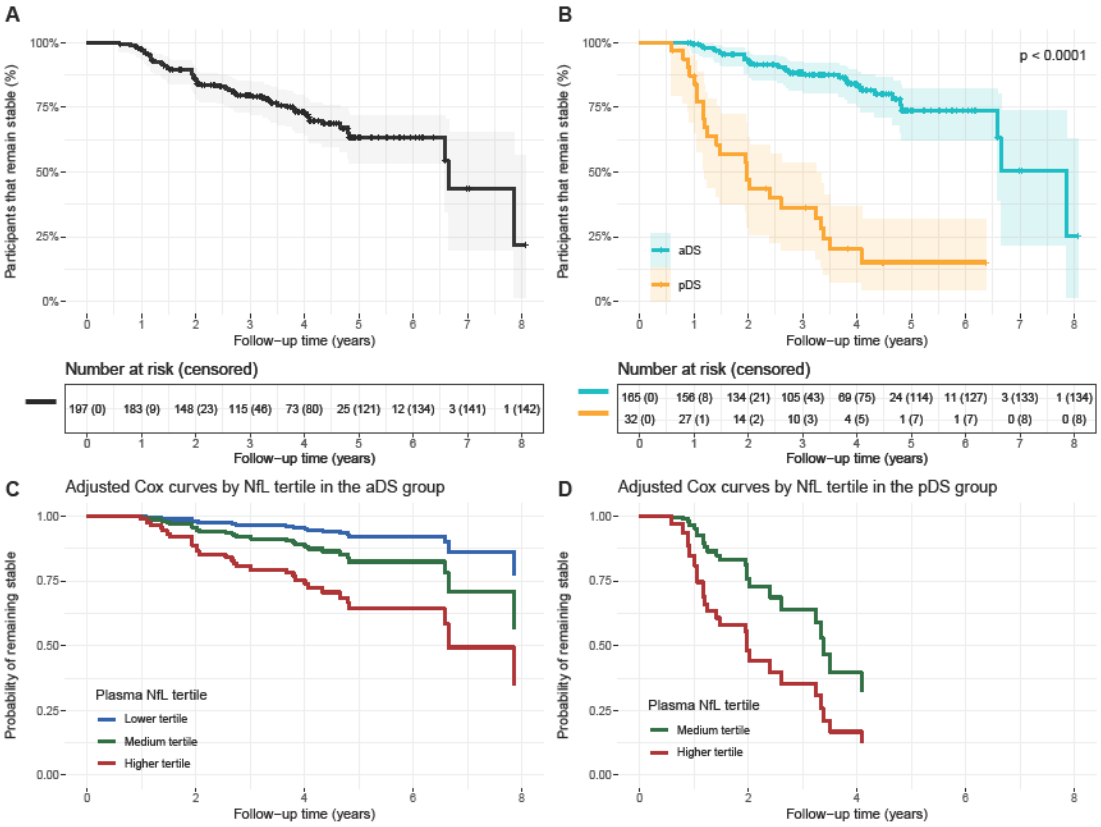

Figure 3: **Clinical progression of participants without dementia and association with baseline NfL concentrations**

Kaplan-Meier curves in all participants (A) and by clinical diagnosis (B). Shaded areas indicate 95% CIs. Predicted progression through adjusted Cox curves for participants in the asymptomatic Down syndrome group (C) and in the prodromal Alzheimer's disease group (D) according to baseline NfL concentrations. NfL=neurofilament light chain.

participants in the asymptomatic and prodromal Alzheimer's disease groups were subclassified as progressors and non-progressors (figure 1B). Similar differences were found when raw NfL concentrations or adjusted NfL concentrations were compared (appendix pp 9–11).

We used ROC analysis to evaluate the diagnostic performance of baseline plasma NfL concentrations. As shown in figure 2, baseline plasma NfL concentrations showed an area under the curve of 0·83 (95% CI 0·76–0·91) to differentiate participants who were asymptomatic from those in the prodromal group. This value increased to 0·94 (0·90–0·97) in the discrimination between asymptomatic and dementia groups. Overall, plasma NfL concentrations showed an accuracy of 0·88 (0·83–0·93) to distinguish participants who were asymptomatic from those who were symptomatic (prodromal Alzheimer's disease and Alzheimer's disease dementia combined). Two cutoff values, 13·0 pg/mL and 18·7 pg/mL, yielded identical maximum Youden indices to discriminate between asymptomatic and prodromal groups. These cutoffs yielded sensitivities of 0·81 and 0·66, while their specificities were 0·74 and 0·90. A cutoff value of 17·0 pg/mL distinguished asymptomatic from Alzheimer's disease dementia groups with a sensitivity of 0·90 and specificity of 0·86. Two cutoff values, 13·6 pg/mL and 16·9 pg/mL, were also found to yield the optimal balance between sensitivity and specificity to discriminate participants who were asymptomatic from those who were symptomatic (prodromal and Alzheimer's disease dementia combined).

We analysed the association of baseline plasma NfL concentrations with clinical progression along the Alzheimer's disease continuum. 54 (27%) of 197 participants without dementia (asymptomatic and prodromal groups) changed clinical diagnosis during follow-up. As represented in Kaplan-Meier curves (figure 3A, B), the whole sample had a median time to progression of 6·7 years (IQR 4·2), shorter in the prodromal group (2·0 years) than in the asymptomatic group (7·9 years; p<0·0001). We studied the association between baseline plasma NfL concentrations and the risk of progression through a multivariable Cox regression analysis. Including age, sex, and intellectual disability as covariates, and diagnosis as categorical predictor, an increase of 1 pg/mL in baseline NfL-adjusted concentrations was associated with a 1·04-fold risk of clinical progression (95% CI 1·01–1·07; p=0·0034). For graphical representation of the adjusted Cox curves (figure 3C, D), participants were categorised into three tertiles according to their baseline plasma NfL concentrations and using the asymptomatic Down syndrome group tertile cutoffs.

We did linear mixed-model analysis to compare longitudinal changes in plasma NfL concentrations between diagnostic categories and to evaluate the association of these changes with clinical progression.

As displayed in figure 4, we found that changes in longitudinal concentrations of plasma NfL differed between clinical categories and progression status (p<0·0001). Plasma NfL concentrations showed an annual increase of 3·0% (95% CI 0·4–5·8) per year in the group of asymptomatic non-progressors, not significantly different from that of participants who were prodromal Alzheimer's disease non-progressors. However, we found an increase of 11·5% (4·9–18·5; p=0·020) per year in the asymptomatic progressors group and 16·0% (8·4–24·0,

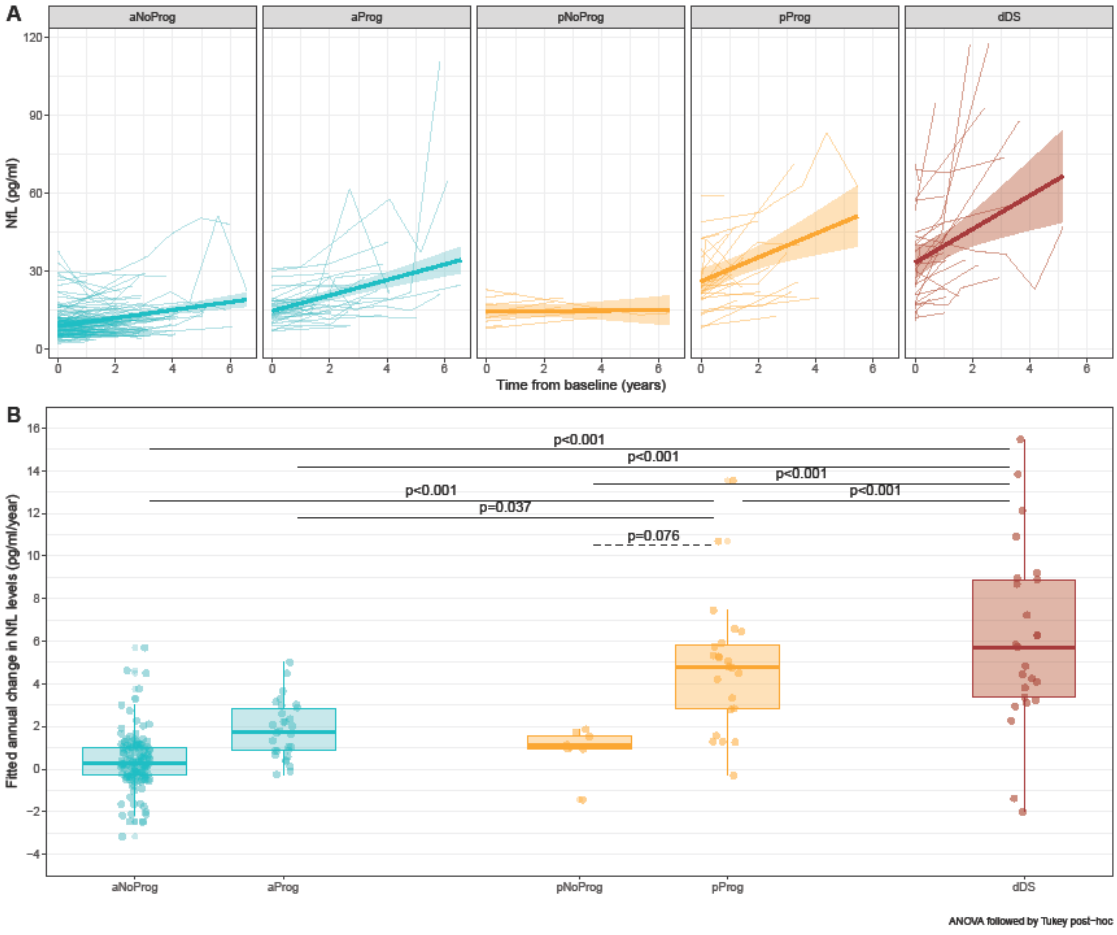

Figure 4: **Trajectories and estimation of the annual increase in plasma NfL concentrations**
Trajectories (A) and annual increase (B) are shown. ANOVA followed by Tukey post-hoc was used for analysis.

p=0·0015) per year in prodromal Alzheimer's disease progressors group. In participants with Alzheimer's disease dementia, NfL concentrations increased by a mean of 24·3% (15·3–34·1; p<0·0001) per year. We found similar trajectories when the analysis was done using W-score-adjusted values (appendix p 15).

DISCUSSION

This longitudinal study of a large multicentre cohort of people with Down syndrome confirms that plasma NfL concentrations are a useful biomarker for the diagnosis of symptomatic Alzheimer's disease in Down syndrome and have good prognostic performance. Moreover, the characterisation of longitudinal trajectories of NfL in plasma showed that the rate of change in plasma NfL concentrations sharply increased along the Alzheimer's disease continuum. These longitudinal changes, which did not seem to plateau along the Alzheimer's disease continuum, posit plasma NfL concentrations as a particularly suitable biomarker for dementia diagnosis and as a surrogate marker of efficacy in clinical trials for Alzheimer's disease in Down syndrome.

The positive association of plasma NfL concentrations with age is consistently found in sporadic Alzheimer's disease,[7,23–25] autosomal dominant Alzheimer's disease,[11,14] and Down syndrome.[6,15,26] In this respect, in a large multimodal biomarker study, we found that in people with Down syndrome, plasma NfL concentrations differ from those in non-trisomic controls at 30 years, 20 years before this difference occurs in patients with symptomatic Alzheimer's disease.[4] We also found differences in relation to the level of intellectual disability such that more severe or profound disability is associated with higher concentrations of NfL. We believe that this finding might relate to the difficulties derived from the clinical assessment of individuals with severe and profound intellectual disability, which might delay their Alzheimer's disease diagnosis. In sporadic Alzheimer's disease, no association of NfL concentrations with educational level has been described.[27]

Baseline plasma NfL concentrations differed between diagnostic groups replicating previous single-centre, crosssectional studies.[4,6,26] The excellent diagnostic performance for plasma NfL in our large multicentre cohort across five countries, using a commercially available assay, reinforces the clinical relevance of this biomarker, as it can be easily and rapidly used by multiple centres effectively. We also showed that plasma NfL concentrations accurately identified patients with prodromal and Alzheimer's disease dementia with Down syndrome, confirming the excellent diagnostic performance of cross-sectional NfL concentrations to detect symptomatic Alzheimer's disease in Down syndrome.[6]

To study the prognostic performance of baseline plasma NfL concentrations, we classified participants as progressors or non-progressors according to changes in their clinical diagnosis during the follow-up. Higher baseline NfL concentrations were associated with clinical progression. Previous studies in small single-centre cohorts of participants with Down syndrome report that higher plasma NfL concentrations predicted the likelihood of dementia and were associated with decreased adaptative behaviour scores at follow-up.[17,26] Similar results were observed in a study in sporadic Alzheimer's disease, in which high plasma NfL concentrations were associated with longitudinal cognitive decline and Alzheimer's disease-related brain atrophy.[7] However, in the same study and others, baseline NfL concentrations did not predict whether patients with mild cognitive impairment would progress to Alzheimer's disease dementia or remain stable.[7,28] Our findings highlight the role of baseline concentrations of NfL, not only as a diagnostic biomarker, but also as a prognostic marker for Alzheimer's disease-related cognitive impairment in Down syndrome.

The understanding of the role of biomarker changes to predict clinical progression is important to monitor the effect of disease-modifying drugs in clinical trials. The longitudinal changes of plasma NfL concentrations were

different across the clinical groups and progression statuses. Plasma NfL concentrations showed an annual increase of 3·8% in the asymptomatic non-progressors group, but the estimated annual increase ranged from 11·5% in those who were asymptomatic progressors to 24·3% in participants with Alzheimer's disease dementia. In sporadic Alzheimer's disease, greater rates of plasma NfL increases are described among people with mild cognitive impairment than healthy controls and among patients with Alzheimer's disease dementia than controls and in people with mild cognitive impairment.[27] In our study, the annual rate of change in NfL concentrations was highest in the Alzheimer's disease dementia group, suggesting that it does not reach a plateau at this stage. A study from 2019 in autosomal dominant Alzheimer's disease, using serial NfL measurements, found that the NfL annual rate of change distinguished mutation carriers and non-carriers almost a decade earlier than NfL concentrations measured at a single timepoint.[12] Similarly, in our study, although crosssectional data did not identify asymptomatic progressors, longitudinal changes did. The increase in longitudinal plasma concentrations in Down syndrome is in contrast to flattening of the curve of estimated annual increases that has been described in longitudinal studies measuring CSF total tau and p-tau181 concentrations in autosomal dominant and sporadic Alzheimer's disease.[24,29–31] However, this finding is in agreement with the acceleration in the atrophy rates found in MRI along the Alzheimer's disease continuum.[32–34] Future studies should further investigate the relationship between atrophy rates and NfL changes. Thus, the increase in the annual change along the Alzheimer's disease continuum in Down syndrome, without evidence for a plateau, facilitates the modelling and power analysis for the use of NfL concentrations as a surrogate marker of efficacy in clinical trials. The advantages in identifying surrogate biomarkers in blood are evident. Plasma NfL concentrations are an easily accessible and inexpensive tool compared with others currently used, such as lumbar punctures, PET scans, or centre-specific neuropsychological assessments.

The major strength of our study is that we established plasma NfL concentrations in a large, well characterised, multicentre population of people with Down syndrome, to our knowledge the largest to date, with a centralised analysis. However, our study also has some limitations. The clinical diagnosis of cognitive decline in Down syndrome, especially in the prodromal stages, is particularly challenging. This factor adds to the difficulty in assessing intellectual disability homogeneously across centres. Formal evaluations of intellectual disability show floor effects (ie, a proportion of participants perform poorly in baseline assessments, making it impossible to identify differences in impairment among the individuals at the low level between people or over time) and might be affected by Alzheimer's disease cognitive decline, making them unreliable in people who are symptomatic. The heterogeneity in the cognitive evaluation protocols between different sites does not allow for thorough examination of associations between plasma NfL concentrations and cognitive measures. Instead, we used clinical diagnosis by expert consensus at each site with masking to biomarker results. The advantage of such a strategy is that it supports the external validity and generalisability of our results, as the diagnosis of prodromal Alzheimer's disease or Alzheimer's disease dementia in Down syndrome is still based on clinical consensus and not on specific sets of cognitive testing scores.[18] Another limitation is the relatively short followup time, but even in this short time we were able to detect relevant differences. Furthermore, the clinical follow-up of the participants in this study is still active, as of May, 2021, at each centre, and the next few years will provide additional and more accurate prognostic results. Finally, as our study did not include additional biochemical and structural Alzheimer's disease biomarkers, we could not analyse the relationship between markers of different pathophysiological processes, which should be considered in future studies.

In summary, our study confirms the clinical utility of plasma NfL for the diagnosis and

prognosis of symptomatic Alzheimer's disease in Down syndrome. The increases in the annual rates of change along the Alzheimer's disease continuum support the use of plasma NfL as a theragnostic marker in clinical trials.

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Supplmentary Figures 12 and 13

Diagnostic and prognostic performance and longitudinal changes in plasma neurofilament light chain concentrations in adults with Down syndrome: a cohort study

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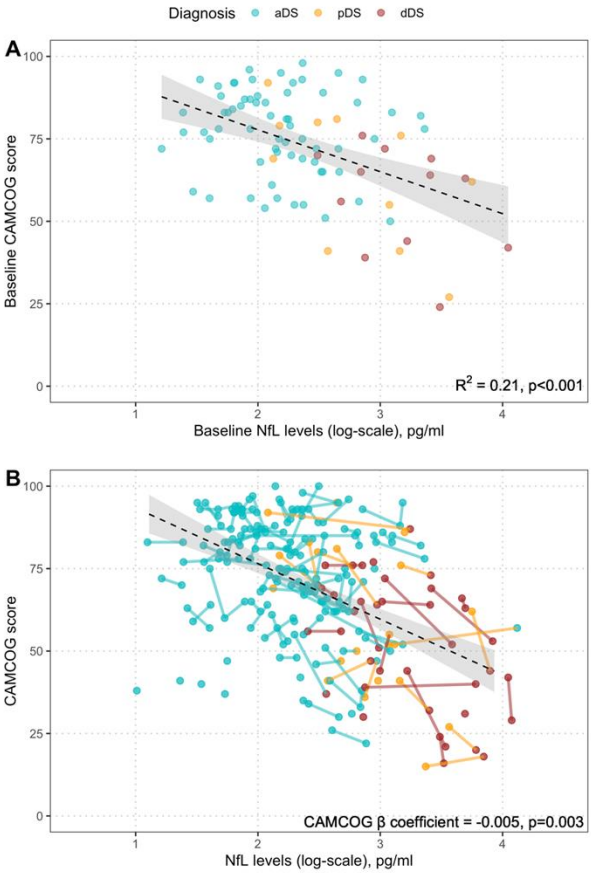
10. Association between plasma NfL levels and cognition

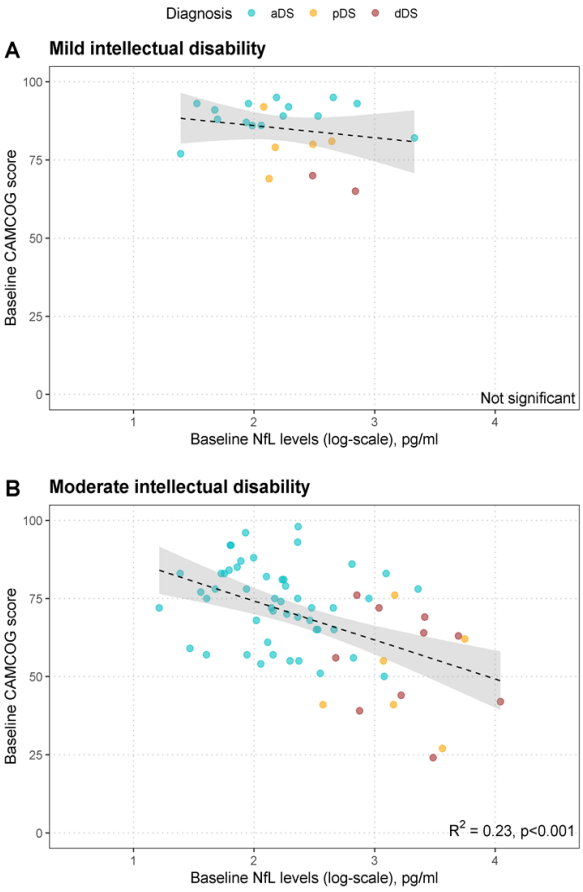
A total of 99 participants had available CAMCOG scores at the time of baseline plasma. There was a significant correlation between baseline plasma NfL levels and baseline CAMCOG scores (panel A).

There were 112 subjects with more than one evaluation for a total of 347 CAMCOG scores. Panel B displays the results of the association between baseline and longitudinal NfL levels and CAMCOG scores in a linear mixed-model that also included diagnosis, intellectual disability, age and sex as fixed factors. We included a random intercept for centre to account for inter-center variability and random intercepts and slopes at the participant level to account for repeated measures.

Supplementary figure 12.



Supplementary figure 13.



We assessed the correlation between baseline CAMCOG score and baseline NfL levels after stratification by level of intellectual disability. The correlation was significant in the group with moderate intellectual disability only (R^2=0.23, p<0.001). (Suppplementary figure 12)

VAMP-2 is a surrogate cerebrospinal fluid marker of Alzheimer-related cognitive impairment in adults with Down syndrome

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SUMMARY

Background: There is an urgent need for objective markers of Alzheimer's disease (AD)-related cognitive impairment in people with Down syndrome (DS) to improve diagnosis, monitor disease progression, and assess response to disease-modifying therapies. Previously, GluA4 and neuronal pentraxin 2 (NPTX2) showed limited potential as cerebrospinal fluid (CSF) markers of cognitive impairment in adults with DS. Here, we compare the CSF profile of a panel of synaptic proteins (Calsyntenin-1, Neuroligin-2, Neurexin-2A, Neurexin-3A, Syntaxin-1B, Thy-1, VAMP-2) to that of NPTX2 and GluA4 in a large cohort of subjects with DS across the preclinical and clinical AD continuum and explore their correlation with cognitive impairment.

Methods: We quantified the synaptic panel proteins by selected reaction monitoring in CSF from 20 non-trisomic cognitively normal controls (mean age 44) and 80 adults with DS grouped according to clinical AD diagnosis (asymptomatic, prodromal AD or AD dementia). We used regression analyses to determine CSF changes across the AD continuum and explored correlations with age, global cognitive performance (CAMCOG), episodic memory (modified cued-recall test; mCRT) and CSF biomarkers, CSF Aβ42:40 ratio, CSF Aβ1-42, CSF p-tau, and CSF NFL. Pvalues were adjusted for multiple testing.

Results: In adults with DS, VAMP-2 was the only synaptic protein to correlate with episodic memory (delayed recall adj.p = .04) and age (adj.p = .0008) and was the best correlate of CSF Aβ42:40 (adj.p = .0001), p-tau (adj.p < .0001), and NFL (adj.p < .0001). Compared to controls, mean VAMP-2 levels were lower in asymptomatic adults with DS only (adj.p = .02). CSF levels of Neurexin-3A, Thy-1, Neurexin-2A, Calysntenin-1, Neuroligin-2, GluA4, and Syntaxin-1B all strongly correlated with NPTX2 (p < .0001), which was the only synaptic protein to show reduced CSF levels in DS at all AD stages compared to controls (adj.p < .002).

INTRODUCTION

Alzheimer's disease (AD) is the leading cause of death in adults with Down syndrome (DS), with a cumulative incidence that exceeds 90% in the seventh decade [1–4]. Current standard cerebrospinal fluid (CSF) markers for AD in the DS population are restricted to surrogate markers of amyloidosis (Aβ42:40 ratio, Aβ1-42) and taumediated neurodegeneration (p-tau) and neurofilament light chain combined with neuropsychological assessment. However, neuropsychological assessment can be confounded by substantial inter-individual variation in intellectual disability (ID). Therefore, there is an urgent need for objective markers of AD-related cognitive impairment in people with DS to improve diagnosis, monitor disease progression, and assess response to disease-modifying therapies.

Synapse loss is an early event in AD [5] and one of the best pathological correlates of cognitive dysfunction [6–9]. As such, synaptic proteins that show AD-associated changes in biofluids are rapidly gaining attention as potential surrogate markers of AD-related synapse loss and may be informative markers of early AD-related cognitive dysfunction in adults with DS.

Neuronal pentraxin-2 (NPTX2), a protein involved in inhibitory circuit dysfunction [10], is a promising biofluid surrogate marker of inhibitory circuit dysfunction and cognitive decline in sporadic AD [11–13], vascular dementia [14], genetic frontotemporal dementia [15], and Lewy body dementia [16]. We recently reported low CSF NPTX2 concentrations in adults with DS across the AD continuum, which correlated with cortical atrophy and reduced glucose metabolism. However, CSF NPTX2 levels did not correlate with measures of cognitive decline in our DS cohort [17]. In the same study, we also evaluated the glutamatergic receptor, GluA4, and found no association with cognitive measures.

The aim of this study was to evaluate a comprehensive panel of alternative synaptic proteins (Calsyntenin-1, Neuroligin-2, Neurexin-2A, Neurexin-3A, Syntaxin-1B, Thy-1, VAMP-2) as surrogate markers of early AD related

cognitive decline in non-trisomic cognitively normal controls (n = 20) and a large cohort of adults with DS (n = 80) from across the preclinical and clinical AD continuum, exploring their relationship to cognitive performance. The panel comprises 8 proteins that were shown to be specifically expressed at the synapse in human frontal cortex postmortem tissue and show CSF alterations that precede clinical symptoms and markers of neurodegeneration in sporadic AD [18]. We also compare the CSF profile of the synaptic panel proteins in adults with DS to that of previously published data on NPTX2 and GluA4 in the same cohort [17].

MATERIAL AND METHODS

Objectives: The primary objective of this study was to evaluate a comprehensive panel of synaptic proteins as surrogate markers of early AD-related cognitive decline in adults with DS from across the preclinical and clinical AD continuum, specifically exploring their relationship to cognitive performance and AD biomarkers.

Study design: This is a single-center, cross-sectional study of CSF levels of synaptic markers in adults with DS, sporadic AD patients and cognitively normal controls. The study (IIBSP-BMS-2018-103) was approved by the local ethics committee (Comité Ètic d'Investigació Clínica, Fundació de Gestió Sanitària de l'Hospital de la Santa Creu i Sant Pau) and was conducted in accordance with the Declaration of Helsinki. All participants gave their written informed consent to participate in the study. Non-trisomic controls were selected from the Sant Pau Initiative in Neurodegeneration (SPIN) cohort, a prospective longitudinal cohort at Hospital Sant Pau, Barcelona, Spain [19]. Adults with DS were selected from the Down Alzheimer Barcelona Neuroimaging Initiative (DABNI), a prospective longitudinal cohort, linked to a population-based health plan in Catalonia, Spain, led by the Fundació Catalana Síndrome de Down and Hospital de Sant Pau [20]. Inclusion criteria for controls required the absence of a cognitive or neurological disorders and normal CSF core AD biomarker (Aβ1-42, Aβ42/40 ratio, t-tau, p-tau) concentrations using our validated cut-offs for sporadic AD [21]. For adults with DS, inclusion criteria for participation in the study required that all participants were over 18 years of age. Where consent was given, participants received a comprehensive neurological and neuropsychological evaluation [22] and underwent a lumbar puncture to assess CSF biomarkers [20]. As in previous studies [4, 20], participants with DS were classified by neurologists and neuropsychologists, blind to biomarker data in a consensus meeting into asymptomatic AD (aDS), prodromal AD (pDS), and AD dementia (dDS) according to previously published criteria [20].

Neuropsychological assessment: The level of ID in adults with DS was categorized according to the Diagnostic and Statistical Manual of Mental Disorders (DSM), Fifth Edition, as mild, moderate, severe, or profound ID, based on caregivers' reports of the individuals' best-ever level of functioning and the Kaufmann Brief Intelligence Test (KBIT) [23]. As previously described [20, 22], neurological and neuropsychological examination of the full range of cognitive impairment included a semi-structured health questionnaire (Cambridge Examination for Mental Disorders of Older People with Down Syndrome and others with intellectual disabilities [CAMDEX-DS]) [24] and a neuropsychological battery including the Cambridge Cognition Examination (CAMCOG) adapted for intellectual disabilities in DS participants and was restricted to those with mild and moderate ID. The Spanish version of the cued recall test modified for use in people with ID (mCRT) [25] was used to evaluate episodic memory as previously described [26]. The total mCRT scores for immediate recall were calculated as free recall score + cued recall score.

CSF collection, biomarker assessment: CSF samples were collected following international consensus recommendations [27] as previously described [28]. Samples had been previously stored at − 80 °C and had not been thawed prior to analysis. Commercially available fully automated immunoassays were used to

determine levels of CSF Aβ1-42, Aβ1-40, NFL, total tau, and p-tau at threonine residue 181 (Lumipulse Aβ1-42, Aβ1-40, total tau G, p-tau 181, Fujirebio-Europe, NFL Simoa Quanterix, MA, USA) [21].

Targeted liquid chromatography mass spectrometry (LC-SRM): We monitored a set of 22 proteotypic peptides corresponding to 10 proteins (Calsyntenin-1, GluA2, GluA4, Neurexin-2A, Neurexin-3A, Neuroligin-2, Syntaxin-1B, Tenascin-R, Thy-1 and VAMP-2) using the previously described selected reaction monitoring (SRM) method [18]. Briefly, we digested individual CSF samples overnight and spiked isotopically labeled peptides (Pepotech SRM custom peptides, grade 2, Thermo Fisher Scientific) into each sample. We analyzed an equivalent of 5 µl of each sample in a randomized order over a 120-min gradient (0–35% ACN + 0.1% FA) in SRM mode using a triple quadrupole-Qtrap mass spectrometer (5500 QTrap, Sciex, Masachussetts) coupled to a nano-LC chromatography column (300 µl/min, 25-cm C18 column, 75 µm I.d., 2 µm particle size). We ran BSA technical controls between each sample. We used isotopically labeled peptides as internal standards. We visualized and analyzed transitions using Skyline 3.5 as previously described [18]. To evaluate the stability of the peptides over the course of the experiment, we injected a pool of all the samples over the duration of the mass spectrometric measurements and monitored the peak area of the standard peptides. The GluA2 peptide was unstable and removed, thus resulting in the exclusion of GluA2 from the study. We processed the SRM transitions using the dataProcess function of MSstats v3.5 package in R [29] and removed transitions with between-run interference (betweenRunInterferenceScore< 0.8). One censored transition (VAMP-2 peptide) where endogenous log2 intensity was below the detection cut-off designated by the MSstats package (8.49) was removed. We used the EqualizeMedians function to normalize the transitions and Tukey's Median Polish to generate a summarized value of transitions for each protein. Two peptides (Calsyntenin-1 and Neurexin-3A) were excluded from the summarization as the endogenous peptide was not detected in all samples. The results for the two Tenascin-R peptides are not reported here due to the lack of synapse specificity of Tenascin-R [18]. Data for the 3 GluA4 peptides from the same SRM experiment have been reported previously [17].

Statistical analysis: All statistical analyses were performed in R version 3.4.3 [30]. We excluded 1 data-point each for Neurologin-2, Neurexin-3A, Syntaxin-1B, Thy-1, VAMP-2, NPTX2 ptau, and t-tau as outliers due to violation of the 3 × interquartile range rule. The outlier values were from 3 different samples from the DS group. Group comparisons were performed using $\chi 2$ test, t test, or linear regression. Where regression residuals deviated from a Gaussian distribution (Shapiro-Wilk $p < 0.05$), tests were performed on square root or log2 transformed values, which did not deviate from a Gaussian distribution (Shapiro-Wilk $p > 0.05$). Raw values were used for those sub-analyses. We used Pearson coefficients to assess correlations. However, to account for the ceiling effect of cognitive tests we used Spearman coefficients on raw values to assess correlations with cognitive measures. Linear regressions of cognitive data were performed on raw data as transformations did not improve the distribution. When comparing the association of multiple synaptic proteins, p values were adjusted for multiple testing using the Benjamini-Hochberg method.

RESULTS

Demographics: Table 1 shows the demographic and clinical data for the participants included in the study, which included 20 controls and 80 adults with DS from across the AD continuum (40 aDS, 19 pDS, and 21 dDS). The mean age-at-analysis across the whole study was 44.5 years (standard deviation; SD = 11.2). Compared to controls, the mean age was comparable in pDS (+ 5 years, p = .20) and dDS (+ 5 years, p = .13) but lower in aDS (− 12 years, p < .0001). The male to female proportion was comparable across clinical groups (p = .45). The level of ID in

	Controls	aDS	pDS	dDS
N	20	40	19	21
Age-at-analysis, years	47 (11, 24-64)	35 (9, 22-57)[b]	52 (4, 45-60)	52 (5, 42-62)
% Female	60%	40%	42%	38%
% Mild/ moderate ID	0%	83%	79%	67%
CAMCOG score[a]	NA	80/107 (11, 55-96, n=31)	70/107 (13.8, 41-92, n=11)[c]	59/107 (13.9, 39-87, n=10)[c]
mCRT score (immediate)[a]	NA	35/36 (1.5, 30-36, n=30)	20/36 (11.2, 0-36, n=12)[c]	15/36 (7.9, 0-32, n=11)[c]
mCRT score (delayed)[a]	NA	12/12 (0.9, 8-12, n=31)	6/12 (3.8, 0-12, n=13)[c]	4/12 (3.3, 0-12, n=11)[c]
CSF $A\beta_{42:40}$ ratio	0.11 (0.01, 0.08-0.12)	0.09 (0.02, 0.04-0.12)[b]	0.05 (0.01, 0.03-0.08)[b]	0.05 (0.01, 0.04-0.08)[b]
CSF p-tau pg/ml	36 (8, 22-54)	35 (24, 10-122)	145 (86, 22-304)[b]	158 (82, 31-323)[b]
CSF t-tau pg/ml	243 (57, 167-366)	295 (166, 86-671)	936 (658, 118-2565)[b]	959 (500, 212-1988)[b]
CSF NFL pg/ml	NA	355 (234, 65-1036)	1071 (767, 313-3123)	1387 (832, 627-3957)

the adults with DS was classified as either mild/moderate (78% of cases) or severe/profound (22% of cases), a proportion that was comparable across clinical groups (p = .37). Cognitive tests were restricted to individuals with mild or moderate intellectual disability. As would be expected, cognitive scores were sequentially lower in pDS (CAMCOG; − 11, p = .02, mCRT immediate; − 15, p < .0001, mCRT delayed; − 5, p < .0001) and dDS (CAMCOG − 21 p < .0001, mCRT immediate; − 20. p < .0001, mCRT delayed; − 7, p < .0001) compared to aDS. As previously reported [20], the mean Aβ42:40 ratio (all p < .0001) was lower in all DS groups compared to controls, while mean CSF p-tau and t-tau levels were higher in pDS and dDS compared to controls (all p <.0001). CSF NFL levels were available for DS only and were elevated in pDS and dDS compared to aDS (p < .00001).

CSF VAMP-2 levels show a distinct profile to other synaptic proteins in adults with DS: The synaptic panel analyzed here includes 7 synaptic proteins previously unpublished in this cohort (Calsyntenin-1, Neurexin-2A, Neurexin-3A, Neuroligin-2, Syntaxin-1B, Thy-1, and VAMP-2) and their comparison to 2 synaptic proteins, NPTX2 and GluA4, previously reported in the same cohort [17]. We first sought to determine the degree of correlation between CSF levels of the 9 synaptic proteins. Figure 1 shows that in adults with DS, synaptic proteins, including Neurexin-3A, Thy-1, Neurexin-2A, Calysntenin-1, Neuroligin-2, GluA4, and Syntaxin-1B, were all correlated (pair-wise r = .70 to .96, n = 78-80, p < .0001). They also all correlated with NPTX2 (pair-wise r = .56 to .84, n = 78-79, p < .0001). VAMP-2 showed the weakest correlation with all other proteins (r = .47 to.69, n = 78-80, p <

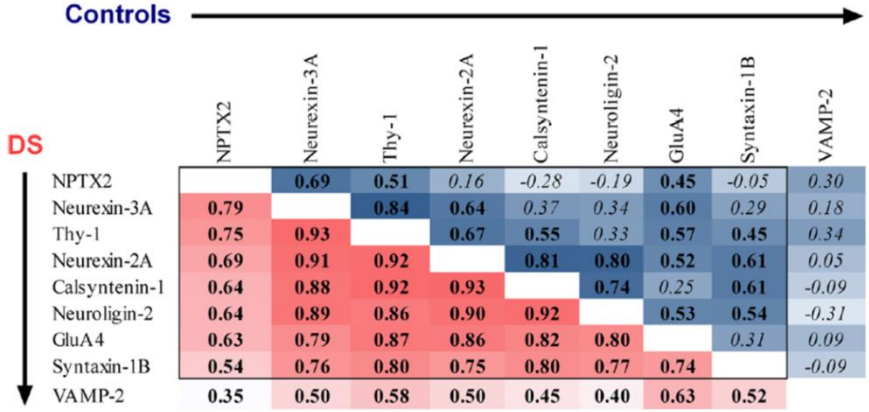

Fig. 1 Pair-wise correlation coefficients of CSF levels of 9 synaptic proteins in DS and controls. r coefficients resulting from statistical tests performed in the DS group (red) and controls (blue) for the 8 synaptic panel proteins and NPTX2 are shown. Degree of shading is relative to size of r coefficients, which are shown in bold where p < .05 and italicized where p > .05. NPTX2 and GluA4 data for these samples are published in [17]

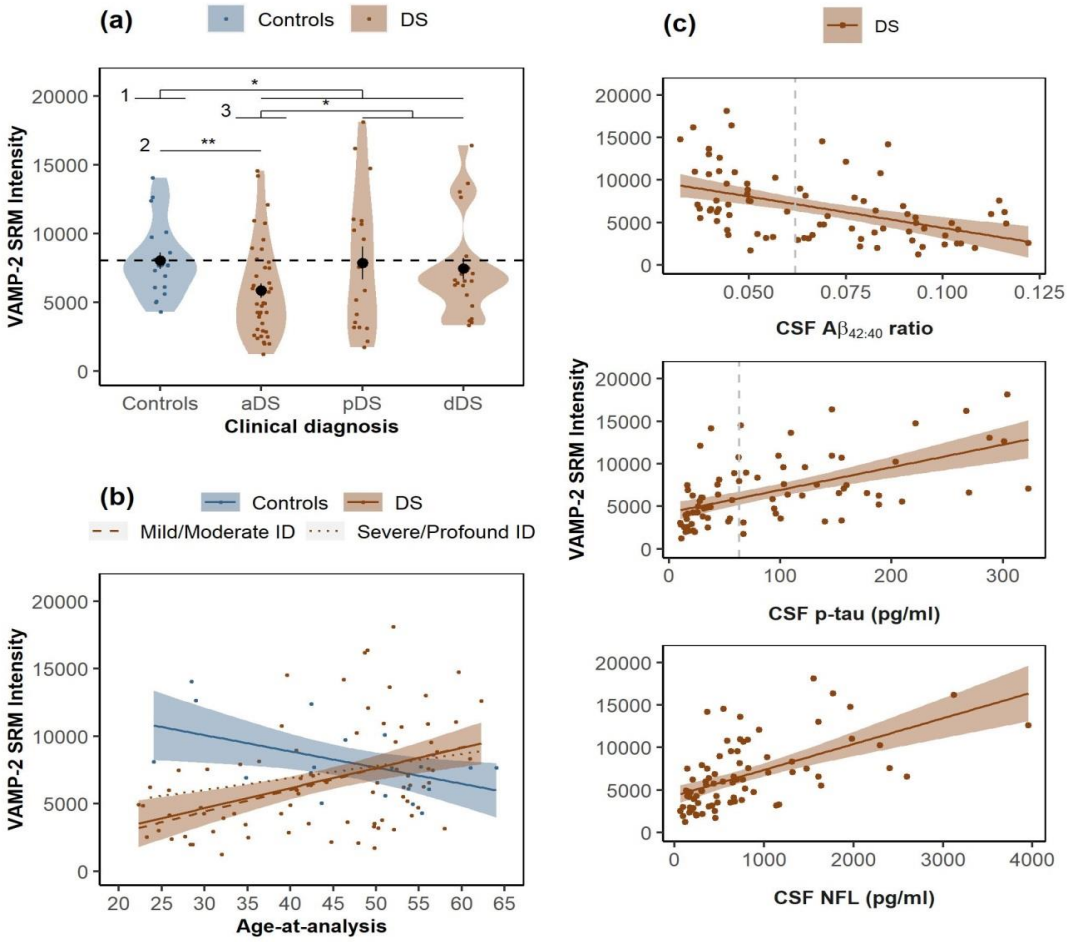

Fig. 2 CSF levels of VAMP-2 in non-trisomic controls and DS. **a** Violin plots show the distribution of SRM intensities for VAMP-2 quantified in CSF for non-trisomic cognitively normal subjects (controls) and adults with DS across AD stages; asymptomatic AD (aDS), prodromal AD (pDS) or AD dementia (dDS). The horizontal dotted line represents the mean value in controls. *p < .05, **p < .01 for linear regression using square root transformed VAMP-2 levels in 1 DS vs controls, 2 aDS vs controls and 3 pDS/dDS vs aDS. **b** Age-at-analysis (years) is plotted against VAMP-2 SRM intensities in controls and adults with DS. **c** CSF biomarkers; $A\beta_{42:40}$ ratio, p-tau and NFL are plotted against VAMP-2 SRM intensities in adults with DS. Linear regression lines in **b** and **c** are shown for each group (see legends). Shaded areas represent standard error of the regression lines. The vertical dotted lines in **c** represent the validated cut-offs for biomarker positivity in sporadic AD

.0001). In controls, all proteins showed weaker pair-wise correlations than in the DS group, although NPTX2, Neurexin-3A, Thy-1, Neurexin-2A, Calysntenin-1, Neuroligin-2, GluA4, and Syntaxin-1B were moderately correlated in at least one pairwise combination (pair-wise r = .46 to .87, n = 20, p < .04). VAMP-2 did not correlate with any other protein in controls (pair-wise r = -.34 to .29, n = 20, p > .14). We took VAMP-2 forward for further analyses due to its relative independence from NPTX2.

CSF VAMP2 changes over the course of AD and with age in adults with DS: Figure 2a shows that mean CSF VAMP-2 SRM intensities were lower in individuals with DS compared to controls (.84-fold, p = .04). Mean CSF VAMP-2 intensities were lower in the aDS group compared to controls (.73-fold, p = .01) and compared to the symptomatic group (pDS and dDS combined; .67-fold, p = .007). CSF VAMP-2 intensities were comparable to controls in pDS (.98-fold, p = .52) and dDS (.93-fold, p = .52). This relative increase in CSF VAMP-2 at late AD stages in adults with DS is supported by Fig. 2b, which shows that CSF VAMP-2 directly correlated with age in DS (r = .43, n = 79, p < .0001). This association was also observed in a linear regression analyses adjusting for degree of ID (adj.r2 = .16, n = 79, p < .002). Conversely, CSF VAMP-2 inversely correlated with age in controls (r = −.51, n = 20, p = .02). The control and DS regression lines for VAMP-2 were non-overlapping at the earliest age included in the study (22 years old) and did not intercept until the age of 42. CSF VAMP-2 was not associated with AD diagnosis when controlling for age p = .61).

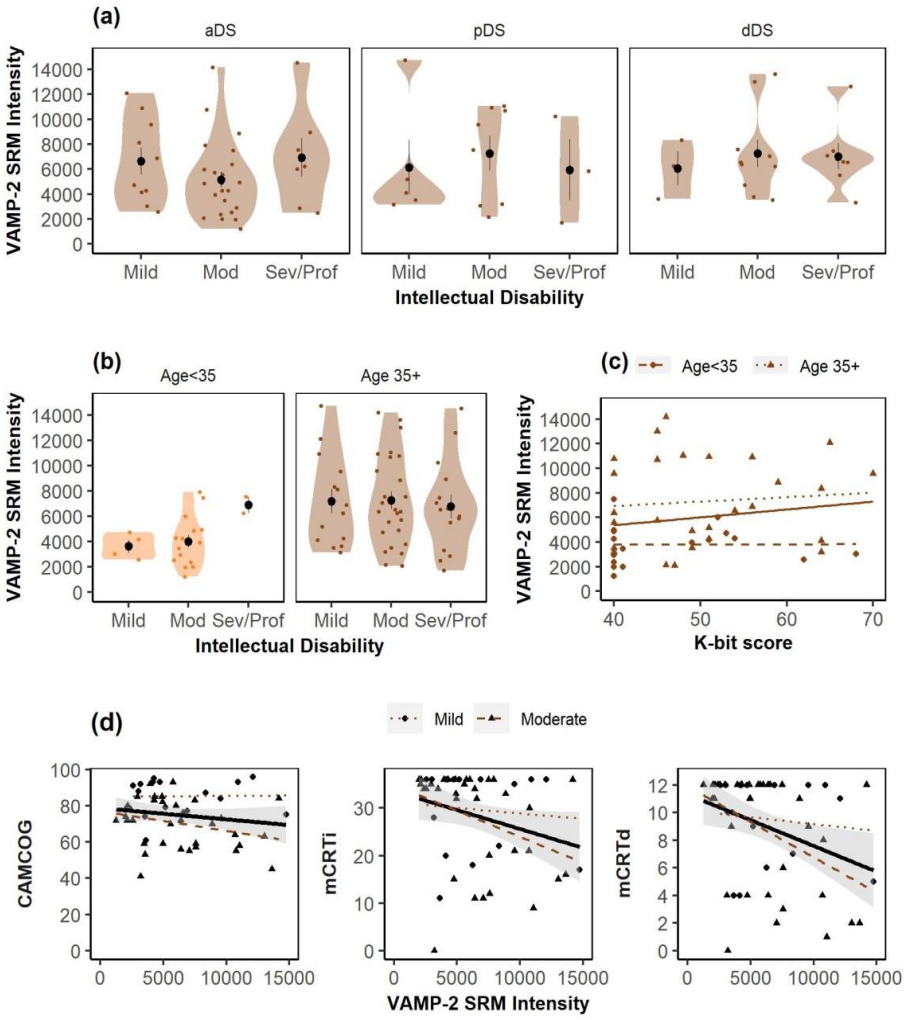

Fig. 3 Relationship between CSF VAMP-2 and measures of intellectual impairment and cognitive performance in DS. Violin plots show the distribution of CSF VAMP-2 SRM intensities in adults with DS grouped according to degree of intellectual disability and **a** AD diagnosis and **b** age group. Circles represent mean intensities and error bars represent standard error of the mean. VAMP-2 SRM intensities in adults with DS are plotted against quantitative measures of intellectual disability (**c**) and cognitive performance (**d**). Linear regression lines are shown for models in total DS dataset and standard error of the regression lines are shown as shaded region. Regression lines for individuals aged < 35 or aged 35+ (**c**) or with mild or moderate ID (**d**) are also shown (see legends)

Figure 2c shows the correlation between CSF VAMP-2 and CSF biomarkers of brain amyloid and tau pathology and axonal degeneration in adults with DS. VAMP-2 inversely correlated with the Aβ42:40 ratio ($r = -.47$, $n = 79$, $p < .0001$) and directly correlated with p-tau, ($r = .56$, $n = 78$, $p < .0001$) and NFL ($r = .57$, $n = 78$, $p < .0001$). To determine whether low CSF VAMP-2 is related to AD biomarker positivity in asymptomatic DS, we compared CSF VAMP-2 SRM intensities in the aDS group stratified by positivity for CSF Aβ1-42 using our validated in-house cut-offs for sporadic AD. Compared to controls, CSF VAMP-2 SRM intensities were lower in individuals positive for CSF Aβ1-42 (0.67-fold, $n = 17$, $p = .009$) but not in individuals negative for CSF Aβ1-42 (0.78-fold, $n = 23$, $p = .30$). Thus, low CSF VAMP-2 is related to AD biomarker positivity and changes over the course of AD and with age in adults with DS.

CSF VAMP2 is associated with cognitive performance in adults with DS:

We next explored the relationship between CSF VAMP-2 and measures of intellectual and cognitive impairment in adults with DS. Mean CSF VAMP-2 SRM intensities were comparable across individuals with, mild, moderate, and severe intellectual disability

(Fig. 3a) in aDS $p = .31$), pDS ($p = .71$), and dDS ($p = .73$). To determine whether

neurodevelopmental factors may influence CSF VAMP-2 concentrations, we compared VAMP-2 across ID groups stratified by < 35 or 35+ (Fig. 3b). CSF VAMP-2 intensities were comparable across individuals with mild, moderate, or severe/profound ID in the younger (p = .15) and older (p = .87) age groups. Furthermore, CSF VAMP-2 SRM intensities were not associated with K-bit score (Fig. 3c) when analyzed independently (r2 = .02, p = .11) or when controlling for age (r2 = .19, p = .77). While Fig. 3d shows a similar regression line for VAMP-2 with CAMCOG and mCRT scores, correlation analyses showed that VAMP-2 SRM intensities were associated with immediate (r = − .32, p = .02) and delayed (r = − .36, p = .007) recall in the mCRT test but not with CAMCOG scores (r = − .19, p = .17). However, since ID had a greater impact on CAMCOG score than on mCRT in our previous study [22], we performed regression analysis of VAMP-2 and cognitive performance including level of ID as a covariate. We found that both ID (t = − 4.04, p = .0002) and VAMP-2 (t = − 2.05, p = .04) were associated with CAMCOG score (model r2 = .27, p = .00002), while VAMP-2 (t = − 2.61, p = .01) but not ID (t = − 0.69, p = .49) was associated with immediate recall in the mCRT test (model r2 = .10, p = .03). We observed a similar association with delayed recall (VAMP-2; t = − 2.94, p = .005, ID t = − 0.76, p = .45). Therefore, VAMP-2 was associated with both CAMCOG and mCRT score in adults with DS even when controlling for ID. VAMP-2 was not associated with any of the cognitive measures when age was included as a covariate (all p > .43).

Compared to other synaptic proteins, VAMP-2 is the best correlate of cognitive performance, age, and CSF amyloid and neurodegeneration markers in adults with DS: Finally, we compared these findings for VAMP-2 to the other synaptic panel proteins and to NPTX2 and applied a strict adjustment of p values to account for multiple testing. Calsyntenin-1 (p = .03), Neuroligin-2 (p = .02), Neurexin-2A (p = .02), Neurexin-3A (p = .02), and Thy-1 (p = .03) were associated with ID in individuals aged <35, suggesting some influence of neurodevelopmental factors on CSF concentrations of these proteins, albeit that the associations did not survive adjustment for multiple testing (all adj.p < .14). The correlation of VAMP-2 with immediate recall (adj.p = .17) and association with CAMCOG (adj.p = .41) did not survive adjustment for multiple testing. Variables associated with at least one synaptic protein (adj.p < .05) in DS are shown in Fig. 4. The associations of CSF NFL and p-tau with each variable are also shown for comparison. VAMP-2 was the only synaptic protein to correlate with mCRT delayed recall (adj.p = .04) and age (adj.p = .0008) and was the best correlate of CSF Aβ42:40 (adj.p = .0001), CSF p-tau (adj.p < .0001), and CSF NFL (adj.p < .0001). On the other

Assesmment	Statistic	NPTX2	Neurexin-2A	Calsyntenin-1	Neurexin-3A	Thy-1	Neuroligin-2	GluA4	Syntaxin-1B	VAMP-2	NFL	p-tau
mCRTi (ID)	pseudo r^2	**0.15**	**0.20**	*0.09*	**0.19**	*0.14*	**0.22**	**0.17**	**0.13**	**0.42**	**0.87**	**0.85**
mCRTd (ID)	pseudo r^2	*0.04*	*0.08*	*0.03*	*0.10*	*0.05*	*0.11*	*0.08*	*0.06*	**0.25**	**0.54**	**0.53**
CAMCOG (ID)	pseudo r^2	*0.50*	*0.48*	*0.49*	*0.49*	*0.48*	*0.48*	*0.48*	*0.47*	**0.56**	**0.65**	**0.66**
Age	r	*-0.09*	*0.09*	*0.06*	*0.18*	*0.17*	*0.22*	*0.23*	*0.24*	**0.43**	**0.56**	**0.36**
CSF NFL	r	*-0.04*	*0.18*	*0.17*	*0.23*	*0.22*	*0.21*	*0.17*	**0.35**	**0.57**	**1.00**	**0.75**
CSF $A\beta_{42:40}$	r	*0.06*	*-0.11*	*-0.04*	*-0.16*	*-0.18*	*-0.13*	*-0.22*	**-0.29**	**-0.47**	**0.57**	**0.69**
CSF p-tau	r	*0.07*	**0.30**	**0.23**	**0.34**	**0.30**	**0.30**	**0.32**	**0.45**	**0.56**	**0.72**	**1.00**
CSF $A\beta_{1-42}$	r	**0.58**	**0.44**	**0.50**	**0.38**	**0.40**	**0.34**	**0.32**	*0.18*	*-0.01*	**0.29**	**0.22**
aDS vs Ctrl	FC	**0.60**	**0.74**	**0.76**	**0.73**	**0.76**	*0.83*	*0.88*	*0.84*	**0.73**	1.03	0.96
pDS vs Ctrl	FC	**0.50**	*0.65*	*0.65*	*0.68*	*0.66*	*0.80*	*0.97*	*0.86*	*0.93*	**3.11**	**4.05**
dDS vs Ctrl	FC	**0.30**	**0.83**	**0.78**	**0.85**	**0.89**	*0.94*	*0.86*	*1.04*	*0.98*	**4.02**	**4.40**

Fig. 4 Comparison of CSF profile of 9 synaptic proteins in adults with DS. Assessment of the 9 synaptic proteins and their association (adj. r^2 or r coefficients) with immediate and delayed recall in the mCRT test (mCRTi and mCRTd), CAMCOG, age, CSF NFL, $A\beta_{42:40}$, p-tau and $A\beta_{1-42}$ and the fold-change (FC) compared to controls in adults with DS across clinical groups. Associations of NFL and p-tau with the same variables are shown for comparison (red for synaptic proteins, blue for NFL and p-tau). Degree of shading is relative to r coefficients or FC. Values are shown in bold where $p < .05$ and italicized where adjusted $p > .05$ (in the case of synaptic proteins this is Benjamini-Hochberg adjusted p). Quantification of NPTX2 and GluA4 and FC for NPTX2 have been published previously in [17]

hand, NPTX2 was the best correlate of CSF Aβ1-42 (r = .58, adj.p < .0001), showed the greatest fold-change across all AD stages (0.34 to 0.55-fold, adj.p < .002), and was the only synaptic protein to show changes in pDS (0.47-fold, adj.p = .002). NFL and p-tau remain the best correlates of cognitive performance in this population and were not altered in the aDS group compared to controls (1.03 fold-change, p = .56 and 0.96 foldchange, p = .93).

DISCUSSION

Here, we report a comprehensive evaluation of synaptic proteins in CSF from adults with DS across the whole clinical continuum of AD. We show that of the 9 synaptic proteins evaluated, VAMP-2 is the only correlate of cognitive performance and age in this relatively understudied population. We also show that while mean CSF VAMP-2 levels were lower in asymptomatic adults with DS compared to cognitively normal controls, mean VAMP-2 levels were elevated at advanced AD stages. Increased CSF VAMP-2 correlated with low CSF Aβ42/40, increased CSF p-tau and NFL and worse cognitive performance. Thus, changes in CSF VAMP-2 are closely related to CSF AD biomarkers and cognitive measures in adults with DS.

In controls, CSF VAMP-2 levels decreased with age and when compared across similar ages, VAMP-2 levels were lower in adults with DS compared to controls from the earliest age included in the study (22 years old) and did not converge until the age of 42. This finding suggests a distinct CSF profile of VAMP-2, and potentially a different mechanism of synaptic pruning, between healthy aging and the presence of AD pathology and/or triplication of chromosome 21. It is possible that the relatively lower CSF VAMP-2 levels in younger adults with DS compared to controls is a result of reduced VAMP-2 expression from birth due to neurodevelopmental factors. However, several lines of evidence suggest that CSF VAMP-2 levels change as a function of AD as opposed to ID: (a) the association between CSF VAMP-2 and age was independent of ID, (b) CSF VAMP-2 was comparable to controls in adults with DS negative for the CSF amyloid marker, (c) the findings reported here for this genetically determined form of AD replicate the nonlinear CSF profile of the 8 synaptic panel proteins previously reported across disease stages in sporadic AD [18], and (d) CSF VAMP-2 did not correlate with K-bit score and was comparable between individuals classified as having mild, moderate, severe, or profound intellectual impairment across all AD stages an in individuals aged < 35 (where AD pathology is less likely to be a confounding factor), but did correlate with age, AD biomarkers, and episodic memory performance. Based on these findings, we propose that low VAMP-2 levels in these individuals may at least partially reflect changes related to the preclinical phase of AD, similar to that previously report in sporadic AD where CSF VAMP-2 levels were nominally reduced in preclinical AD and significantly elevated in prodromal and dementia stages compared to cognitively normal controls [18].

We have previously evaluated NPTX2 as a synaptic marker in the same cohort reported here [17]. Similar to VAMP-2, CSF NPTX2 levels were lower in DS compared to controls, albeit that NPTX2 was reduced at all AD stages. In fact, here we report that compared to the other 8 synaptic proteins, NPTX2 was the only protein to be reduced at all AD stages compared to controls. However, unlike VAMP-2, CSF NPTX2 did not correlate with cognition or age. The distinct expression and function of these two proteins could explain their distinct CSF profiles in adults with DS. VAMP-2 is expressed at the human cortical synapse with increased enrichment compared not only to the other 7 synaptic panel proteins evaluated here but also to the widely used pre-synaptic marker, synaptophysin [18]. This high synapse specificity supports other studies that have shown that VAMP-2 is predominantly found at glutamatergic synapses [31] as part of the synaptic exocytosis core vesicular complex [32] where it is necessary for regulating the releasable pool of glutamate at the pre-synapse [33] and is also critical for post-synaptic trafficking of glutamate receptor subunits, particularly in the CA1 region of the

hippocampus [34]. Reduced VAMP-2 brain expression has been reported in AD [35]. NPTX2 is specifically expressed by pyramidal neurons where it mediates activity-dependent strengthening of pyramidal neuron excitatory synapses on GABAergic parvalbumin interneurons [10]. Therefore, both VAMP-2 and NPTX2 are specifically expressed at distinct populations of synapses where they have distinct functions that are critical for synaptic transmission. We therefore propose that CSF levels of these 2 synaptic proteins may reflect degeneration of distinct synapse populations. While NPTX2 remains a promising surrogate marker of inhibitory circuit dysfunction in AD, DS, and other neurodegenerative diseases, VAMP-2 may be a better surrogate marker of cognitive performance in adults with DS.

A previous study reported that CSF NPTX2 correlates well CSF levels of two other synaptic proteins, SNAP-25 and neurogranin in sporadic AD CSF [12]. In this study, we report that, with the exception of VAMP-2, CSF levels of synaptic proteins were highly inter-correlated in adults with DS and that VAMP-2 was the only synaptic protein not to correlate with at least one other synaptic protein in controls.

The novelty of VAMP-2 is that it was the only synaptic protein evaluated here to correlate with age (a surrogate measure of disease progression in DS) and mCRT in the DS population. Similar to our previous study, we found that ID had a greater impact on CAMCOG score than on mCRT [22] such that the association of VAMP-2 with mCRT score was evident without the need to control for level of ID. The mCRT test is a version of the CRT modified for use in DS and discriminates well between DS adults with and without dementia [26]. The

CRT test is considered a clinical marker of episodic memory disorders due to medial temporal damage, especially in the CA1 field of the hippocampus [36], which is consistent with the functional role of VAMP-2 at CA1 synapses [34].

Study limitations: While DS and autosomal dominant cohorts with available CSF are scarce, further replication of the findings reported here in independent genetic AD and DS cohorts would be valuable. A limitation of this study is the cross-sectional design, particularly in the analysis of cognitive decline. Longitudinal studies are needed to fully establish the prognostic value of VAMP-2 in DS cohorts.

Conclusion: NFL remains the best CSF correlate of cognition in this population and this work opens the door to future studies exploring the prognostic capacity of CSF VAMP-2 in adults with DS and sporadic AD. The data reported in this manuscript show proof-of-concept for CSF VAMP-2 as a potential marker of synapse degeneration that correlates with CSF AD and axonal degeneration markers and cognitive performance. Whether VAMP-2 could be a useful addition to NFL to specifically monitor synapse engagement and therapeutic response, particularly in AD clinical trials would be an interesting avenue worth pursuing; as anti-tau and anti-Aβ are common therapies, there is a need for an alternative surrogate measure of cognitive performance not directly affected by the drug. An ELISA-based immunoassay to facilitate the quantification of VAMP-2 in patient CSF is in development. Moreover, as VAMP-2 is also detectable in blood [37], whether plasma VAMP-2 can be used as a surrogate marker of brain VAMP-2 is also worth pursuing.

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GENERAL DISCUSSION

This doctoral thesis is based on data from the Down Alzheimer Barcelona Neuroimaging Initiative (DABNI) cohort. DABNI is the largest cohort worldwide of adults with DS with multimodal Alzheimer disease (AD) biomarker studies. It aims to better characterize the clinical and cognitive course of AD in this population and explore the performance of different fluid biomarkers in plasma and CSF.

The diagnosis of prodromal AD and AD dementia in individuals with DS is a challenge mainly due to the premorbid ID. From a neuropsychological perspective and prior to this doctoral thesis, the variable levels of ID had impeded the development of validated cutoffs even when using the few instruments to assess cognition that have been validated in this population. In the paper included in the annex of this thesis (annex 1), we studied the diagnostic performance of two adapted neuropsychological tools to detect symptomatic AD in DS, the CAMCOG-DS and the mCRT, and we proposed, for the first time, cutoff points employing two different approaches. We derived both normative data from cognitively stable adults (similar to the approach used in the general population) and thresholds derived from the ROC analyses. These tests showed good diagnostic performance when individuals were stratified by the level of ID, but only in individuals with mild and moderate ID. This paper was the cornerstone of the first work of this doctoral thesis, which aimed to characterize the longitudinal AD-related clinical and cognitive changes in this population.

This first work estimated, for the first time, the risks of progression at 1, 3 and 5-year follow-ups along the AD continuum in the different age-ranges, and the longitudinal cognitive changes by the level of ID and by clinical group when using the above-mentioned adapted neuropsychological tests. Importantly, annual longitudinal change was similar in mild and moderate ID, suggesting that, although required for diagnosis, stratification by the level of ID might not be necessary for monitoring cognitive changes over time. We also explored the presence of practice and floor effects that should be considered when analyzing and interpreting neuropsychological data in both clinical practice and clinical trials.

The second work focused on the study of the diagnostic performance of two adapted neuropsychological tests to detect cognitive decline in adults with DS. Specifically, we compared the diagnostic performance of the cross-sectional and longitudinal evaluations to diagnose prodromal AD and AD dementia in adults with DS. Specialists on ID typically recommend longitudinal assessments to diagnose dementia in individuals with ID, as single-point evaluations are deemed inaccurate due to the variable level of ID. Contrary to our expectations, cross-sectional cognitive assessments outperformed longitudinal change at 1 year. This suggests that cross-sectional rather than intra-individual (short term) cognitive performance is preferable for diagnosis of prodromal AD and AD dementia in individuals with DS when using suitable neuropsychological tools applied by experienced neuropsychologists.

The two last works of this thesis explored the relationship between cognitive performance and biochemical biomarkers in plasma and CSF. We studied the diagnostic and prognostic performance of plasma NfL levels as well as their correlation with cognitive performance. Finally, we explored the performance of more novel biomarkers. Specifically, the fourth work was, to our knowledge, the first comprehensive evaluation of synaptic proteins in CSF in adults with DS across the whole clinical continuum of AD. We found that CSF VAMP-2 levels might be a potential biomarker for AD-related cognitive decline in this population.

The data presented in this thesis has focused on the diagnosis of symptomatic AD and the study of the natural history of clinical, cognitive, and biomarker changes of AD in adults with DS. In this discussion we will first discuss the implications of our works on the care of adults with DS and the impact it might have in the design of both health plans for adults with DS and clinical trials against AD in this population. Finally, we will outline some limitations in our work and future lines of investigation to continue developing the DABNI cohort to advance our knowledge of AD in DS.

"Every year on his birthday you start thinking, 'This is the best he is going to be,'" "I look for any little slip. I watch him sign his name and if he lifts his pen differently I think, 'Oh, it's Alzheimer's disease.'"
Taffy. 78 yo. Jay's mother, a person living with DS[130]

1. Risk and penetrance of AD in adults with DS

At the beginning of this thesis, there were limited and inconsistent epidemiological data about the incidence, prevalence, and cumulative risk for symptomatic AD in DS. The reported prevalence of dementia, cumulative incidence (or risk), or age of onset were variable, and it

was deemed that many individuals with DS would not develop the clinical manifestations of AD despite the universal presence of the neuropathological hallmarks of the disease by the age of 40. These discrepancies between studies stemmed from a combination of the difficulty in diagnosis, inadequate sensitivity in assessment criteria, and the use of different diagnostic methods in single-center studies,[2] but most importantly, from selection and survival biases. For instance, the cumulative risk of symptomatic AD in this population increases exponentially with age and reaching 90-100% by the end of the seventh decade of life[114,131] irrespective of the level of ID.[132] A recent metanalysis performed in the Alzheimer-Down Unit established a very consistent onset of AD dementia in DS at a mean of 53.8 (95%CI, 53.1-54.5) years. Contrary to a widespread assumption, that metanalysis demonstrated that the variability in the disease onset was very similar in AD in DS and ADAD. Furthermore, that study demonstrated that the mortality data was consistent with near full penetrance (and AD being the leading cause of death of this population accounting for approximately 70% of deaths). This near full penetrance is in agreement with the universal AD pathology and the data from the longitudinal studies with long follow-up periods.[133,134] However, longitudinal studies assessing the risk of AD in DS are scarce; most of them have limited sample sizes, and or do not include direct cognitive measures or biofluids biomarkers.

The large sample size in our first study enabled us to estimate, for the first time, the incidence or risk of progression along the AD continuum at different age ranges and different follow-up times, 1, 3 and 5 years. Longitudinal progression along the AD continuum showed a clear age dependency in asymptomatic individuals, starting at the age of 40 and progressively increasing to 57.5% in those older than 50 years after 5 years of follow-up. This age dependency was not seen in patients with prodromal AD, who universally progressed to AD dementia after the 3-5 years of follow-up. Our results demonstrate the ultra-high risk of developing symptomatic AD in adults with DS, and the almost inevitable progression to dementia once the clinical symptoms emerge. These results are aligned with recent studies, supporting an overall low rate of dementia in people with DS younger than 40 years, but with an exponential increase in the incidence and prevalence thereafter, reaching 88–100% in those older than 65 years.[115]

Progression estimates to symptomatic AD in the general population are variable, mainly due to different study settings, different definitions of progression, and whether or not (and which) AD biomarkers were included. Thus, Petersen 2016 reported an annual overall progression from MCI to AD dementia of 8-15%, when biomarkers were not assessed, but the risk increased to 30% at one year in those MCI with positive AD biomarkers.[135] Similarly, Vos et al. 2013 described the 5-year progression in cognitively healthy individuals to symptomatic AD

according to the Preclinical Working Group of the NIA-AA criteria in a cohort of 311 cognitively normal volunteers (CDR=0) with a mean age of 72.9 [sd 6.0]. There was a 2% progression rate in the group with negative biomarkers (Stage 0), 11% for those in stage 1 (amyloidosis), 26% in stage 2 (amyloidosis + neurodegeneration) and 56% in stage 3 (subtle cognitive decline + amyloidosis + neurodegeneration).[136] The progression in the stage 3 group with positive biomarkers for AD is comparable to our data in adults with Down syndrome, where 57.5% of adults with DS over 50 years (an age at which all individuals have full blown AD pathology) developed symptomatic AD. In short, in the general population, AD biomarkers distinguish first a subgroup with an underlying AD pathology from those without. Depending on the AT(N) combination, it is possible to infer to a certain degree where in the AD continuum the subject is. However, risk estimates cannot be as accurate as in genetically determined AD, where the most salient feature shared by autosomal dominant AD and DS is the near full penetrance of symptomatic AD[114,131,133,137] at a relatively young age.

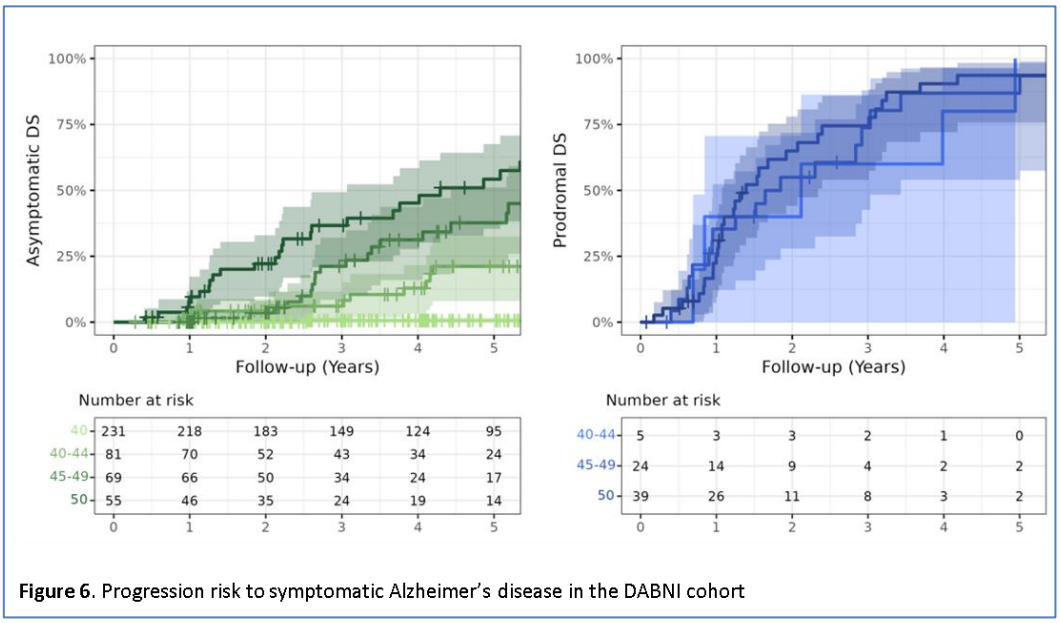

Figure 6. Progression risk to symptomatic Alzheimer's disease in the DABNI cohort

In addition to the influence of age in the development of symptomatic AD, we also explored ID as a possible risk factor for an earlier progression along the AD continuum. We found similar rates of clinical progression to symptomatic AD in individuals with mild, moderate, severe, and profound ID, suggesting no impact on the clinical progression of the disease. However, there is still inconclusive evidence on this matter in people with DS.[138] It has been proposed that individuals with more severe ID experience a faster rate of cognitive decline with a lower survival time from dementia diagnosis.[31] Nonetheless, other studies found no association

between cognitive decline and ID level in agreement with our current results.[139,140] These variable results may be justified by the different sample compositions. In particular research studies are usually biased towards less severe levels of ID.[140] To avoid this bias, we included all levels of ID in the survival analysis and obtained a representative sample from a well characterized cohort of adults with DS that may allow a better generalization of the results.

In sporadic AD and ADAD, a higher cognitive reserve is associated with a delay in the onset of cognitive decline and faster cognitive decline after onset.[141] Cognitive reserve is defined as "the adaptability of cognitive processes that explain differential susceptibility of cognitive abilities to brain aging and/or damage".[142] The results in the general population suggest that the mechanism by which cognitive reserve mediates the relationship between pathology and cognitive function is by delaying the onset of symptoms, rather than reducing the rate of cognitive decline.[143] This issue in individuals with ID is more complex because there is a mixture of biological and environmental factors that, in some instances, are difficult to distinguish. On the one hand, more severe ID seems to not be clearly related to higher incidence or prevalence. However, some studies with small sample sizes have suggested that environmental interventions aimed at improving the level of intellectual functioning may be useful in deferring the onset of dementia in adults with DS.[144] However, the impact of cognitive reserve in age of onset and progression of AD in DS is understudied and needs further clarification.

In short, this doctoral thesis builds on previous results by our group and others providing evidence of the ultra-high risk of symptomatic AD in DS and the almost inevitable course of the clinical manifestations of dementia.[114,131,133] Age is tightly associated with progression during the preclinical stage of the disease independently of ID level, but this age-dependency was not seen in patients with prodromal AD, who universally progressed to AD dementia after 3-5 years of follow-up. The main difference between these progression rates and those of the general population with positive biomarkers for AD is the age at which symptom onset manifests, which is >20-30 years younger in DS. Given the high lifetime risk of AD dementia in this population and the almost inevitable progression to symptomatic AD, there is a need to better characterize the clinical picture of AD in DS, to define reliable diagnostic criteria as well as to study biomarkers, not only for early diagnosis but also for prognosis and disease monitoring, health planning and clinical trials.

"I have to help find a cure for Alzheimer's to help other people with Down syndrome and old people like my mom."
Binek. 35 yo. Person living with DS[130]

2. Clinical diagnosis of Alzheimer's disease in Down syndrome

The clinical diagnosis of symptomatic AD in people with DS is difficult mainly due to the coexistent ID, which is highly variable across individuals. As mentioned in the introduction, shortly before the beginning of this thesis, the National Task Group Consensus Recommendations for the diagnosis of dementia in individuals with ID emphasized the need for a stepwise and comprehensive assessment of suspected cognitive decline. Among other suggestions, this group of experts recommended including at least one standardized tool for cognitive assessment to generate a baseline score that could be tracked over time to capture within-person dementia-related cognitive decline.[78] Moreover, they stressed the ineffectiveness of population norms when assessing an individual with ID for dementia.

The works included in this thesis (and in the annex) support the good diagnostic performance of two adapted neuropsychological tests for the diagnosis of symptomatic AD in a large population-based cohort of adults with DS: the CAMCOG-DS, and the mCRT. In particular, the work in the annex shows that, as in the general population, diagnosis of prodromal AD and AD dementia can reliably be done in adults with DS based on the observation of low levels of cognitive test performance relative to DS population norms, when appropriately stratified by the level of ID. This stratification allowed us to provide several neuropsychological cutoff points for prodromal AD and AD dementia using different approaches. At the beginning of this thesis, due to the questioning of the validity of population norms for symptomatic AD diagnosis, only a few specialized groups worldwide routinely used neuropsychological assessment in the diagnostic process. Consequently, diagnosis of dementia in this population was mainly based on informant-based interviews, which assess behavioral symptoms and activities of daily living beyond direct neuropsychological assessments.[77] This was in stark contrast with the approach used in the general population, in which the neuropsychological assessment to objectively measure cognitive impairment is the basis for the diagnosis of mild cognitive impairment (or prodromal AD) and dementia. Furthermore, objective cognitive impairment in the general population, which is the cardinal clinical symptom of dementia, is ascertained using population norms to establish cutoff points. The work in the annex, which laid the foundation for the two first papers of the thesis, defined, for the first time, cutoff points based on normative data from young adults with DS (age≤35) in mild and moderate ID separately. We also provided cutoffs based on the separation between asymptomatic individuals and both prodromal AD and AD dementia patients using receiver-operating

characteristic (ROC) curve analyses, an approach that had been used in previous studies in DS, including the original validation of the Spanish version of CAMCOG,[110] and lately in the original and French validation of the CRT.[111,120] However, the cutoffs derived from the ROC analyses were not congruent across studies. Our study and the recent work by Krinsky McHale et al. derived higher cutoffs from the ROC analysis than the Spanish and French validation. As shown in the second work included in this thesis, ROC analyses are very sensitive to sample composition (i.e. samples of symptomatic individuals with different disease severity) and this, might explain some of these discrepancies. The inclusion of prevalent vs. incident patients and the inclusion of more advanced AD stages lower the cutoffs in this approach. As an alternative, we propose population norms might be more stable, and are thus preferred in the general population. Our work demonstrated, for the first time, the feasibility of such an approach in DS, but future works in other cohorts must confirm its robustness, which would increase the diagnostic confidence and greatly enhance multinational collaborations.

The first paper included in this thesis further studied the age-related cognitive decline associated with different stages of the AD continuum. In contrast to the great impact of ID in the cross-sectional cognitive assessment, the longitudinal cognitive decline did not differ in those individuals with mild and moderate levels of ID, both in global cognition and memory performance. These results suggest that stratification by ID is mandatory for symptomatic AD diagnosis but might not be necessary to monitor disease progression in clinical practice or clinical trials (we will delve more into this point later in the discussion). In our study, progressive cognitive decline was observed in some individuals as early as 35-40 years that is about 15 years before the estimate age of dementia onset, as previously reported.[116,145] Early cognitive decline starting years before dementia onset has also been reported in the general population[146] A recent study compared the cognitive performance in sporadic AD from the National Alzheimer Coordinating Center (NACC) and ADAD from the dominantly inherited Alzheimer network (DIAN) before and after symptom onset. Mutation carriers declined more slowly during the preclinical stage and performed better at symptom onset than those with sporadic AD; however, after symptom onset, mutation carriers declined more rapidly.[147] Although further research is needed, the qualitative interpretation of the results of our first study (Figure 1 and Figure 3 in the supplementary material) suggests a similar pattern of cognitive decline to that in ADAD, with mild progressive decline in the preclinical stage and a more severe impact of AD pathology after diagnosis. These results suggest sporadic AD, ADAD and AD in DS represent similar AD pathology with some particularities. The cognitive decline observed in sporadic AD at onset of the preclinical stage may be multifactorial, with

contributions from other co-occurring conditions besides AD. However, the more aggressive course of ADAD and AD in DS after symptom onset, despite the younger age of these individuals, suggests some additional or different pathological processes. For instance, the greater amyloid burden in ADAD and DS compared to sporadic AD may contribute to greater cognitive decline after symptom onset, but the exact mechanism is still unknown.[147]

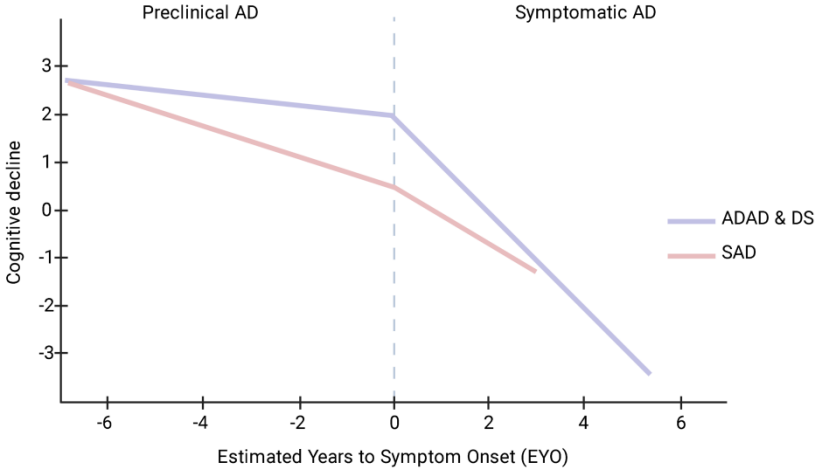

Figure 7. Conceptual model of cognitive decline progression along the AD continuum in sporadic Alzheimer's disease and in Autosomal dominant forms of AD including DS.

Adapted from Buckles et al. 2021. *Created with BioRender.com*

In the second paper of this doctoral thesis, we further studied the diagnostic performance of cross-sectional and annual longitudinal cognitive decline measures for the diagnosis of symptomatic AD in adults with DS. Contrary to our expectations, the one-year longitudinal assessment was less useful than single-point evaluation for the diagnosis of AD in DS when the individuals were stratified by the level of ID. This unexpected result may be justified by several factors, most importantly, the intra-individual cognitive variability with repeated assessments. This variability with repeated measurements is not specific of DS, it is also found in the general population. Day-to-day variability in mood, fatigue and stress can significantly impact participants' attention and thus influence in cognitive performance.[125,126] Of note, the 2 and 3-year longitudinal assessment yielded similar results to the baseline evaluation due to the higher signal to noise ratio (or power of the 2-year change over the intra-individual longitudinal variability) and the fall at longer follow-ups was even superior. Other factors that might contribute to the lower performance of the one-year longitudinal assessments include the practice effects that increased the variability and the ceiling and floor effects observed in

the memory test mCRT at different stages of the disease, which also reduced the power of the longitudinal assessments. These results are in agreement with those in the general population, where longitudinal cognitive decline did not offer substantial benefit over cross-sectional assessment in detecting preclinical AD or incident MCI in the general population. The increased test-retest variability among subjects limited the utility of longitudinal interpretation despite using advanced statistical methods to account for intra-individual variability.[128,148]

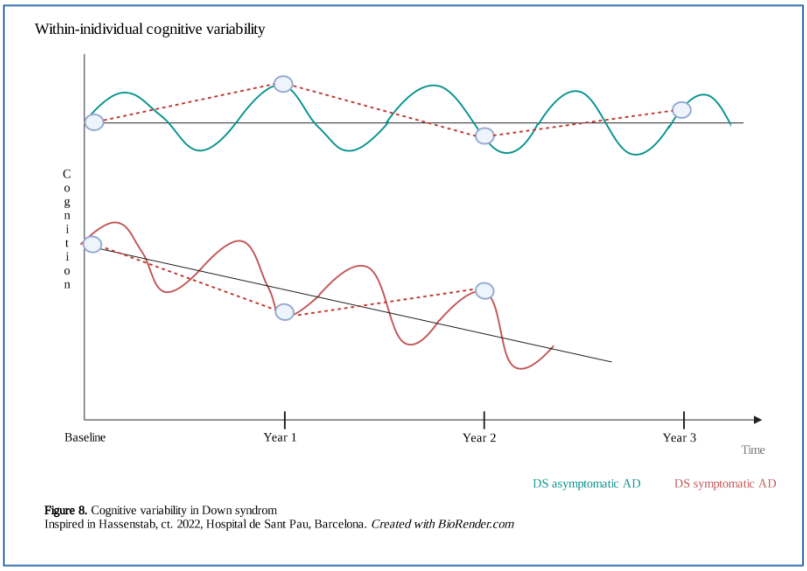

Figure 8. Cognitive variability in Down syndrom
Inspired in Hassenstab, ct. 2022, Hospital de Sant Pau, Barcelona. *Created with BioRender.com*

The practice, ceiling and floor effects deserve further comment. We found practice effects due to repeated measurements up to two years after the baseline evaluation. We would like to note that we did not alternate the different sets of stimuli of the mCRT as some stimuli in set B were deemed difficult or less common in our country than set A. However, this is a limitation in our protocol and a formal validation study should be performed to confirm this clinical impression. These practice effects were only seen in young (<40yo) asymptomatic individuals and were most prominent in episodic memory. Curiously, practice effects in memory were seen until the age of 40, despite the progressive decline with age seen on the baseline evaluations. CAMCOG-DS showed more discreet practice effects until the age of 35. From a neuropsychological perspective, it is known that practice and ceiling effects can obscure the objectivity of cognitive decline during follow-up in clinical practice and led to a misinterpretation of the cognitive measurement. However, the absence of practice effects in episodic memory have been proposed as an early marker of AD in the preclinical stage themselves[149] and deserve further investigation in DS. Importantly, practice effects might be a source of variability in the forthcoming preventive clinical trials, which most likely will recruit

both de novo participants and those already followed in longitudinal trial-ready cohorts, leading to reduced effect sizes.[150,151]

Floor effects were also more prominent in the mCRT than in CAMCOG. These floor effects obscured the acceleration in the cognitive decline along the AD continuum. Thus, progression along the AD continuum was associated with faster cognitive decline when subjects were adjusted by the baseline cognitive scores, but not if not adjusted. Floor effects have previously been described in DS when using non-adapted neuropsychological tools, but not in the specific context of AD-related cognitive decline. To our knowledge, no previous studies have analyzed practice or ceiling effects in DS in the context of AD-related cognitive decline, but have been repeatedly identified in individuals with MCI or AD dementia in the general population, with a reduction of practice effects in episodic memory in preclinical AD.[149] It has also been demonstrated that these issues can interfere with the results of clinical trials, leading to low or medium effect size range[150] and that they can influence the sample size estimation for clinical trials.[151] As mentioned, the mCRT was more sensitive than the CAMCOG to detect prodromal AD in DS. In this sense, some tests used to assess cognitive decline in the general population also showed this limitation due to ceiling effects. In short, as in the general population memory performance (e.g. mCRT in DS and FCSRT in the general population) changed earlier and was more sensitive than global cognition, but the later (e.g. CAMCOG in DS or ADAS-Cog in the general population) has a larger dynamic change and might be more appropriate to track change in symptomatic individuals. The most widely applied cognitive test in the general population, the MMSE, has also ceiling, practice, and floor effects for assessing cognitive decline in the general population and in relation to AD pathology.[152] The maximum MMSE score can easily be reached by cognitively unimpaired individuals showing ceiling effects, which is particularly frequent among individuals with a high educational level.[152] Conversely, most patients in an advanced stage of AD reached the minimum MMSE score, making impossible to measure their real cognitive performance (floor effects). This defines curvilinearity. The MMSE has been shown to be a highly curvilinear psychometric test, as its sensitivity to change varies strongly, with a very poor sensitivity to change in high scores (27–30) and a relatively good sensitivity to change in the medium range of scores (10–20)[153] Similarly, the FCSRT also showed ceiling and floor effects in the general population. The TIR measure of the FCSRT in highly educated patients has ceiling effects, suggesting that it is less appropriate than FIR to assess the earlier cognitive changes in subjects with high cognitive performance. Indeed, the Free measure of the FCRT showed less ceiling effects, but earlier floor effects, suggesting a different dynamic range.[154] In short, it is essential to understand the

dynamic ranges of the tests and when the scores become curvilinear in order to properly track cognitive decline in this populations.

General recommendations for controlling practice effects in serial testing include the incorporation of control or placebo groups, longer test-retest intervals (which is not so feasible in the context of AD clinical trials), and, importantly, using alternate tests forms with equivalent sets of stimuli, or a dual baseline assessment approach.[155] A dual baseline approach requires the administration of the cognitive tools twice before the study; if the most prominent improvement occurs from the first to the second assessment, this second score may serve as a baseline for subsequent assessments.[156] The other classical alternative to account for learning effects is to use different validated sets of stimuli from the same test, especially when assessing memory. Regarding the statistical control of the learning effects, some approaches have been developed to try to distinguish when a change in performance in an individual is clinically meaningful or it reflects change due to practice effects based on reliable change index calculations, hierarchical linear modeling, or regression models.[156]

In summary, our results show not only similar diagnostic and prognostic performances for the diagnosis of symptomatic AD, but also similar psychometric properties when using formal neuropsychological evaluations in DS (with adapted tests), sporadic AD and ADAD. Although further studies are needed, our results suggest that, as in the general population, prodromal AD and AD dementia can be diagnosed in DS using neuropsychological tests and AD-related cognitive declined tracked with adapted tests in subjects with mild and moderate levels of ID.

"I'm giving you 30 years to fix this."
Margot, mother of a newborn with DS[130]

3. Alzheimer's disease biomarkers in Down syndrome

Considering the high prevalence of AD in DS and the underlying difficulties for clinical diagnosis, the use of biomarkers to assist in the diagnosis of prodromal AD and AD dementia can be especially useful. The use of biomarkers represents a radical change in the diagnostic approach to the disease; but, they were understudied in the population with DS at the beginning of this thesis. However, important progress has been made in the field of AD biomarkers over the past decade, although longitudinal data is still limited. In this sense, our group and others have described remarkable similarities between AD biomarkers in DS and other populations with sporadic AD and ADAD in agreement with the very similar neuropathological findings across the different forms of the disease.[115,157,158] The natural

history of the AD in DS clinical and biomarker findings has been established.[114] Thus, as in sporadic AD and ADAD, individuals with DS show a long preclinical phase in which biomarkers follow a predictable order of changes during more than two decades before the clinical diagnosis of prodromal AD is established. This sequence of changes starts with early changes in CSF Aβ and plasma NFL by the age of 28/30 years, followed by CSF p-tau increases in the mid-30s and fibrillar amyloid deposition detectable with PET in the late 30s (10 years later than the CSF Aβ changes). In the fifth decade there is hippocampal atrophy in parallel with a decline in cognitive performance.[87,114]

Core AD biomarkers (Aβ1–42, t-Tau, and p-Tau) in CSF are useful for the diagnosis of prodromal AD and AD dementia in DS.[114,137,159] However, despite the great utility of CSF biomarkers for AD, blood-based biomarkers have obvious advantages and can be especially useful in this population. There have been several reports analyzing the plasma Aβ40 and Aβ42 peptides using inmunoassays or with SIMOA in adults with DS[17] These reports have consistently shown elevations in the plasma Aβ40 and Aβ42 peptides when compared to euploid individuals, reflecting the APP gene dose, but with conflicting results with respect the trajectory of changes along the AD continuum and very low diagnostic performances at the individual level. Of note, there are no studies with the more novel mass-spectrometry technique, which has demonstrated good accuracy to detect brain amyloid deposition[102] The diagnostic performance of plasma NfL and p-Tau181 for AD in DS have shown very promising results,[137,160]and novel biomarkers, such as synaptic proteins, are now beginning to be explored in DS in plasma with similarly encouraging results.[161]

In relation to these advances, the last two works included in this thesis aimed to further characterize biochemical biomarkers for AD in DS. Specifically, we assessed the diagnostic, prognostic performance and studied the longitudinal changes of plasma NfL. Finally, we explored, for the first time, a panel of synaptic CSF proteins, and proposed CSF-VAMP2 as a potential biomarker for the early stages of AD in DS with good correlation with cognitive measures.

In our third study, we replicated and confirmed the clinical utility of plasma NfL in a longitudinal multicenter sample.[114,137,162,163] Plasma NfL concentrations accurately identified DS patients with prodromal and AD dementia, confirming the excellent diagnostic performance. Importantly, plasma NfL concentrations showed a good correlation with cognitive measures. These findings reinforce the clinical relevance of this biomarker, which can be easily and rapidly used by multiple centers using a commercially available assay. Increased

concentrations of plasma NfL in sporadic AD have also been reported, even in early stages, showing a good correlation with core AD biomarkers (cognitive tests, neuroimaging, and CSF biomarkers).[97] Similar results have been also described in ADAD.[164] We would like to emphasize that NfL is a non-specific biomarker of neurodegeneration which is also elevated in other neurological diseases in the general population besides sporadic AD.[165] Nonetheless, in the context of DS and ADAD, where other neurodegenerative disorders are anecdotal, plasma NfL represents a promising AD biomarker to be used not only in a research context, but also in routine clinical practice with evident advantages over more sophisticated biomarkers.

In addition to their diagnostic utility, biomarkers can be used for other purposes, such as to identify subjects at high risk to develop the clinical symptoms of AD, or to monitor disease progression. In this sense, we also described the prognostic performance and the longitudinal changes of plasma NfL concentrations in a multicenter cohort of adults with DS. To our knowledge, no previous studies had examined the prognostic performance of NfL levels in this population. In sporadic AD, greater rates of plasma NfL increase are described among people with MCI when compared to healthy controls, and in patients with AD dementia compared to controls or MCI.[97] A longitudinal study in ADAD using serial NfL measurements found that the NfL annual rate of change distinguished mutation carriers and non-carriers almost a decade earlier than NfL concentrations measured at a single time point.[166] Similarly, in our study, cross-sectional data did not identify asymptomatic progressors, but longitudinal changes in NfL concentrations did. The longitudinal trajectory of plasma NfL along the AD continuum showed a stepwise increase in the annual rates of change, even in the dementia stage. Importantly, these results support the use of plasma NfL to track the evolution of AD in DS even in symptomatic stages. The increase in longitudinal plasma NfL concentrations in DS along the different stages of AD continuum is in contrast to the flattening of the curve of estimated annual increases of CSF total tau and p-tau181 concentrations in sporadic AD and ADAD (of note there are no papers showing the longitudinal trajectory of CSF core AD biomarkers in DS).[167]

To validate and demonstrate the clinical utility of an AD biomarker it is also important to study its correlation with neuropsychological measures capturing AD-related cognitive decline. We did a sub-analysis including all those individuals in whom CAMCOG-DS scores were available. We found a significant correlation between baseline plasma NfL levels and baseline CAMCOG-DS scores, as well as between baseline and longitudinal NfL levels and CAMCOG scores. We also assessed the correlation between baseline CAMCOG score and baseline NfL levels after stratification by level of ID. The correlation was significant in the group with moderate

intellectual disability only, probably due to the reduced sample size of the group with mild ID. In 2019, an exploratory study including 12 adults with DS [mean age 45.33 (sd: 8.53)][168] also found a significant correlation between plasma NfL and CAMCOG-DS measures, as well as with the error rate on CANTAB-Paired Associates Learning test. Similar results were observed in a study in sporadic AD, in which high plasma NfL concentrations were associated with longitudinal cognitive decline.[97]

Finally, in this same study, we also explored the NfL concentrations in the different levels of ID. We found that those individuals with a more severe level of ID were associated with higher concentrations of NfL. We hypothesize that these elevated NfL levels could be related to the difficulties derived from clinical assessment of individuals with severe and profound ID, which might delay their AD diagnosis.

As previously mentioned, in addition to the classical core AD biomarkers, recent research has shown the potential of novel biomarkers. Very recently a study including 61 individuals with DS showed higher levels of serum beta-synuclein but not pTau181 in asymptomatic individuals with DS, suggesting an early alteration of beta-synuclein as a marker of synaptic dysfunction, preceding the elevation of pTau-181.[161] In this line, in our last study where we explored a panel of synaptic proteins, we found that CSF VAMP-2 was closely related to CSF AD biomarkers in adults with DS and showed a good correlation with cognitive measures along the whole AD continuum. We suggest that the reduced levels of VAMP2 in the preclinical stage may reflect reduced synaptic density in these individuals who already show signs of brain amyloidosis, an effect that is confounded by widespread neurodegeneration at later disease stages. As in our study in the annex, we found that ID had a greater impact on CAMCOG-DS score than on the mCRT but did not impact CSF VAMP-2, that is, that CSF VAMP-2 levels were comparable across individuals with mild, moderate, or severe/profound in our sample. Only 3 synaptic proteins have been previously studied in DS: neurogranin, NPTX 2, and the nerve growth factor.[169–171] Of these, only one study included cognitive data, but found no correlation between CSF NPTX2 and CAMCOG-DS. Similar to the general population,[98] CSF VAMP-2 levels in our study were lower in adults with DS and preclinical AD as compared to cognitively normal controls but elevated at advanced stages of AD. Studies of different CSF synaptic proteins in ADAD have also suggested that early abnormalities, including synaptic damage and neuronal injury, begin shortly after the beginning of brain amyloid accumulation.

In short, our results provide important data supporting the excellent diagnostic and prognostic performance of plasma NfL, one of the most promising biomarkers to be used in clinical

routine. Plasma NfL has clear advantages over CSF biomarkers, MRI or PET scans and it is almost ready to be implemented in clinical practice. Blood-based biomarkers can be especially useful for those individuals with more severe levels of ID when the cognitive tests are not feasible; however, the influence of ID and other possible confounding factors is still unclear. Due to the novelty of CSF synaptic biomarkers, its clinical use needs to be further studied, our study can be considered as proof-of-concept for CSF VAMP-2 as a potential marker of synapse degeneration.

"Those of us with an extra 21st chromosome have a black cloud hanging over us" "My fear is stepping up to the microphone, and forgetting why I am there, or what I should say. What if I forget how to swim? Or find my way to the pool?"
Gaffney. 43 yo. Person living with DS[130]

4. Implications for health plans and Alzheimer's disease clinical trials for adults with Down syndrome

The works included in this thesis might have important implications for the design of health plan for adults with DS and of clinical trials against AD in this population.

4.1 Implications for health plans for adults with Down syndrome

Improved medical care of individuals with DS over the past decades has led to an increase in life expectancy in this population, changing the epidemiological picture. Currently, almost 50% of individuals with DS are over the age of 40 and survival reaches the seventh decade of life.[5] Thus, there has been an increase in age-related medical needs in this population, most importantly AD. However, there are no specific health plans for adults with DS. Moreover, specialized centers with knowledge on aging and ID are scarce, and health professionals attending to the general population do not feel comfortable to diagnose symptomatic AD in individuals with DS.[1] Finally, families are often unaware of the high risk of developing dementia in this population and the patient referral, when done, is often late. Information on the rate of change over time is valuable for assessing the results of therapeutic interventions, predicting the severity of cognitive decline, and planning for long-term health care.[83]

Thankfully, during the past few years, some international efforts were done to develop consensus guidelines to define standardized assessment practices among health care professionals, including cognitive assessments and multidisciplinary teams to attend to this growing population. Currently, well-trained clinicians with expertise in the diagnosis of AD and

ID can make accurate diagnoses, despite the difficulties in assessing the AD-related cognitive decline, and the absence of validated operatized clinical diagnostic criteria. As mentioned previously, adapted neuropsychological tests are required for a precise cognitive assessment, and population norms were a gap in knowledge that this PhD tried to cover. Another important recommendation derived from our second work is to abandon the need to consider the within-person longitudinal change over the baseline evaluation. Overall, the works included in this thesis have contributed to develop and update clinical guidelines for dementia and DS for the "Spanish Society of Neurology",[172] the "Monterrey Clinical Guidelines"[173] and the "Spanish Health Program for people with Down syndrome".[174]

Importantly, the neuropsychological tests included in our work showed excellent diagnostic performance in the baseline evaluation in individuals with DS with mild and moderate ID, which represent approximately 70% of this population. Nonetheless, there remains 30% of the population with profound to severe ID who cannot benefit from this neuropsychological protocol; hence there is a need to develop additional diagnostic tools for this population. Despite the low benefit of cognitive assessment in those subjects with severe or profound ID, these individuals must also be included in the health plans with specific considerations. Caregivers or reliable informants are essential for the diagnosis of symptomatic AD in DS, independently of their ID level, but especially in the case of individuals with more severe ID. Some efforts aimed at obtaining direct measures of individuals with more severe ID are being developed. For instance, Esteba et al. are currently working on the development and validation of the "Cognitive Exploration Scale for People with Intellectual Disability and Extended Support" (ECDI-SE, for its acronym in Spanish). Other neuropsychological testing, such as the Severe Impairment Battery (SIB), also showed acceptable performance in individuals with more severe ID.[175]

Other direct clinical implications are on the age at which to start the screening for AD-related cognitive decline and the incorporation of biomarkers. The risk of developing dementia before the age of 40 is low, but some individuals do show signs of cognitive decline at age 35[5,31,114,133,176] Population-based health plans to screen for AD should, therefore, start at the latest at age 30/35 to detect those individuals at higher risk to progress to dementia. However, we recommend starting the clinical visits at an earlier age to ensure a reliable cognitive, functional, behavioral and health baseline when the individual is at their highest level of functioning. Despite we've seen that longitudinal cognitive assessments do not ameliorate the baseline diagnostic performance of the neuropsychological tests, we should be cautious until more studies confirm our findings. In any case, from the age of 40 onwards, the risk of

developing symptomatic AD exponentially increases with age and we recommend medical and cognitive follow-ups annually.[83] On the other hand, biomarkers will represent a breakthrough in the diagnosis of symptomatic AD in DS. In the third work we showed the excellent diagnostic and prognostic performance of NfL for AD in individuals with DS using a commercially available assay. Soon blood-based biomarkers will play a major role in our routine clinical practice and may be used as screening tool a primary care prior to the decision to use more sophisticated tests or make referrals to specialized clinics. The usefulness and need of AD biomarkers in clinical trials and in the research context is universally recognized.[10,177,178] However, there are some limitations when attempting to incorporate them into routine clinical practice, especially due to costs, limited validation and experience, and poor access in less developed countries.[179]

Recently, the Alzheimer's Association published some recommendations for the appropriate use of blood biomarkers in AD in the clinical practice in the general population. They recommend to cautiously start using blood-based biomarkers in specialized memory clinics as part of the diagnostic work-up of patients with cognitive symptoms with further confirmation of the results with CSF or PET measures. Additional information as assay validation, cutoff points for each biomarker and different contexts of use (screening, diagnosis, staging, etc.), definition of pre-analytical protocols, and studies examining confounding factors that may affect the interpretation of blood-based biomarkers are needed within large and diverse populations are required before the widespread use in primary care.[178] Low body mass index, cardiovascular disease, and impaired kidney function all impact plasma biomarker levels in the general population,[180] but despite a higher prevalence of several of these factors in DS, their impact in thus population has not been studied. These recommendations should be adapted to the DS context, but future work is needed to answer some of the aforementioned questions in DS.

In brief, adults with DS require health plans to diagnose symptomatic AD. The experience of the Alzheimer-Down Unit during the last 10 years in attending individuals with DS, and the contributions of the works included in this thesis, suggest the clinical identification of this high-risk population, most likely in combination with biomarkers, will provide people with DS and their families or caregivers the opportunity of an early diagnosis, professional counseling, and symptomatic treatment, as well as the opportunity to participate in clinical trials and benefit from the new disease modifying treatments that will most likely be approved in the near future.

4.2 Implications for clinical trials against Alzheimer's disease in Down syndrome

Individuals with DS constitute the largest population of genetically determined AD, due to the triplication of the APP gene located in chromosome 21. The predictable sequence of pathogenic events in DS with a long asymptomatic stage during which disease-modifying interventions might be most effective, opens opportunities for early interventions that, are not possible in sporadic AD. Therefore, individuals with DS are probably the most appropriate population in which to perform preventive or modification clinical trials.

Despite the high prevalence and lifelong risk of AD dementia in individuals with DS, they have been largely excluded from research advances and from AD clinical trials. To date, few pharmacological randomized clinical trials have focused on improving dementia symptoms in people with DS. In 2015, a Cochrane report concluded that there was insufficient evidence to determine whether drugs approved for the treatment of dementia due to AD in the general population were effective in DS (this is cholinesterase inhibitors and the glutamate receptor antagonist memantine).[181]

AD is now possibly the greatest medical challenge we face in the 21st century. The pharmaceutical industry has spent more than 40 years trying to develop a drug experiencing numerous setbacks. The investigation of this disease is an expensive, complex, and high-risk process with a very low rate of success. Importantly, very recent results have revolutionized the field and offered hope for the treatment of AD in the very near future. In June 2021, Aducanumab, which is a monoclonal antibody directed against Aβ, received its first approval in the USA for the treatment of the early stages of AD. However, its results were controversial.[182] In any case, it was the first promising treatment for AD approved by the FDA in almost 20 years. Very recently, in September 2022, a press release regarding the CLARITY AD trial, a Phase III clinical trial using the monoclonal antibody lecanemab, announced that the trial had met the primary (and all secondary) endpoints, showing a 27% statistically significant reduction of clinical decline (using the clinical dementia rating scale sum of boxes) in large global clinical study of 1,795 participants with early AD after 18 months of FUP. Lecanemab (also known as BAN2401) is an intravenously biweekly administered anti-amyloid drug. These (and all) anti-amyloid drugs presented amyloid-related imaging abnormalities (ARIA) as the main side effect, both cerebral edema and microhemorrhages that in most cases were asymptomatic.[183] None of these drugs have been tested in the DS population and specific clinical trials are needed, with a special attention to the occurrence of ARIA due to the more severe amyloid antipathy in this population.[61,184]

There are specific challenges for clinical trials in individuals with ID. These include heterogeneity within the population, inter-individual variability, lack of endpoints to assess efficacy, reliance on informant-based questionnaires, and complexity in the interpretation of findings.[163] To move forward in AD clinical trials for DS, it will be essential to first identify the appropriate sample population and outcome measures. There is a need to define the proper age range of the target population, the premorbid level of ID, and to control the co-occurring health conditions. Second, we will need to describe the safety profile of the experimental drug by studying pharmacokinetics and common adverse events, especially those that may be unique to people with DS. Finally, we will need to find the proper outcome measures reflecting the impact on objective measures of disease progression and presumably clinically meaningful outcomes.[185] It is vital to understand AD as a continuum with a long preclinical stage to decide when to start preventive treatment.

Safety is essential in any pharmacological trial. It is important to conduct phase I studies including individuals with DS to study safety, tolerability, toxicity and the possible influence of their co-occurring conditions because there may exist pharmacokinetic and pharmacodynamics differences between individuals with DS and the general population. Very recently results of a multicenter double-blind placebo-controlled dose-escalation phase 1 randomized clinical trial were published including 16 adults with DS. In this study, the ACI-24 vaccine (a monoclonal antibody targeting amyloid peptides) was safe and well tolerated in DS, it showed evidence of immunogenicity and target engagement was observed with any adverse events.[186] Finally, we need not only to explore new therapies, but also to perform phase IV clinical trials with approved drugs in the general population.

Regarding the study design and sample population, the results included in this thesis provide relevant information. First, we provided the progression rates along the AD continuum at 1, 3 and 5 years of follow-up and by age ranges. Our data confirm that a preventative trial would have high statistical power and might be also useful to define the optimal age range of the target populations. An important finding is that individuals with mild and moderate ID show a similar pattern of cognitive decline. Therefore, although needed for diagnosis, the participants included in a trial need not be stratified by the level of ID in order to analyze the cognitive endpoint, reducing the sample size needed and the economic cost of the trial.

We further develop the discussion on the advances of this thesis to enable the definition of appropriate cognitive outcome measures and the validation of biomarker candidates for AD clinical trials for the DS population in the next section.

4.2.1 Cognitive outcome measures for Alzheimer's disease clinical trials in Down syndrome

The lack of adequate outcome measures validated in individuals with DS represents a significant obstacle for clinical trials. Outcome measures need to reflect the actions of the drug in the sample selected. However, there is a lack of data regarding the cognitive changes that occur along the AD continuum in DS, and this has contributed to adults with DS being overlooked for inclusion in AD clinical trials. As mentioned, cognitive outcome measures are essential to conduct AD trials. Thus, in 2017, the National Institute of Health (NIH) assembled a group of key opinion leaders to review and identify the appropriate cognitive measures for trials in DS. This group of experts pointed out the importance of selecting precise, sensitive, reliable, and valid measures for the assessment of cognition and behavior, but also highlighted the need for studying floor/ceiling effects of the tests.[187]

The works included in this thesis provide relevant data regarding this guidance. First, we confirm that it is possible to capture and monitor cognitive decline due to AD in the DS population with mild and moderate levels of ID for the duration of a preventive trial. Most patients with prodromal AD were also able to complete the neuropsychological assessment with the CAMCOG-DS at baseline, but the completion rate decrease considerably in the longitudinal follow-ups when they progressed to a dementia stage (of note completions rates were lower for the mCRT). Therefore, mCRT might be better suited for preventive trials whereas the CAMCOG-DS is more appropriate to monitor disease progression.

Importantly, practice effects and floor effects must be considered. Floor effects for some neuropsychological tests can limit feasibility of many cognitive instruments used to assess longitudinal cognitive decline or the impact of the drug. Moreover, practice effects were also found. These should be considered when combining participants de novo and from longitudinal cohorts. On the other hand, practice effects can increase the dynamic range of the tests and, therefore, their power to detect a response to treatment, especially in the context of short trials. When these kinds of effects are ignored, erroneous conclusions can be drawn about the course of diseases and effectiveness of treatment. These findings might explain some of the divergent effects in clinical trials between the cognitive trajectories of the placebo arm and historical longitudinal cohorts in sporadic AD and ADAD,[188] irrespective of the randomization and potential treatment effect.

4.2.2 Alzheimer's disease biomarker in clinical trials for Down syndrome

Biomarkers play a critical role in drug development, not only for the inclusion criteria, but also for measuring the potential efficacy. Without biomarkers, trials rely on behavioral outcomes, which reflect combined effects of many different factors such as learning and environment, and these measures may not be sensitive enough to measure treatment effects, especially in individuals with ID.

As mentioned, very recently, blood-based biomarkers have been proposed as potential biomarkers to improve the design of clinical trials for neurodegenerative diseases in the general population.[178] First, they proposed plasma Aβ42/Aβ40 and p-tau assays with established thresholds, for use as a first screening step in AD trials to subsequently confirm the diagnosis with more sophisticated tests such as PET or CSF samples. However, they highlight the need for further validation before they are used as primary endpoints and the ethical implications of disclosing biomarker results to individuals who are currently asymptomatic.

In DS, the recommendations may be different or be nuanced. First because all individuals with DS are, by definition, in the preclinical state. Second, because the performance (and use) of the biomarkers might vary. For example, the results in our studies suggest the use of plasma NfL are specific in the context of DS (as opposed to sporadic AD).

There have been revolutionary advances in analytical techniques in the field of AD biomarkers and, currently, biofluid tests are more sensitive to detect pathology. Until now, the focus has primarily been on the diagnostic and prognostic performance of AD biomarkers. As additional therapies are developed biomarkers will be progressively incorporated with theragnostic purposes (endpoints), but also to monitor safety or risk stratification.[189] For example, the recommendations of use for these therapies include at least 3 annual MRIs for safety (to monitor for amyloid imaging abnormalities, ARIA) or the use of the APOE haplotypes for risk stratification.[188] In this sense, synaptic biomarkers are expected to facilitate monitoring efficacy.[190] Our data support the use of CSF VAMP-2 as a potential marker of synapse degeneration. It correlates with CSF AD and axonal degeneration markers and cognitive performance, and it could be useful to monitor therapeutic response as an alternative surrogate measure of cognitive performance not directly affected by the drug. Further, this biomarker could be used for a more precise inclusion of participants in clinical trials, mostly in participants with more severe levels of ID, when clinical and cognitive assessment is more complicated. Previously, NPTX2 showed reduced levels in adults with DS compared to cognitively normal, nontrisomic controls, even prior to AD onset. However, this biomarker had

a limited potential as a CSF marker of AD due to its low correlation with cognitive decline in this population.[170] In sporadic AD we have also identified and provided clinical validation for a set of synaptic proteins (Calsyntenin-1, GluR4, Neurexin-2A, Neurexin-3A, Syntaxin-1B, and Thy-1) that can be detected in CSF.[98] These could provide added value in to assess disease progression in individuals at-risk for AD and AD patients, and could improve enrichment and monitoring of drug efficacy in pharmaceutical drug trials.

In essence, finding appropriate outcome measures is crucial for understanding the results of clinical trials, as well as to really capture the effectiveness of the experimental treatment. Hence, finding and developing adapted cognitive and clinical outcome measures is essential to maximize the validity of future clinical trials for the population with DS.[187]

4.2.3 Collaborative efforts for Alzheimer's disease clinical trials for Down syndrome

There are many reasons to be optimistic about the future of AD research in DS. Much progress has been made in the past 10 years regarding AD and DS, partly due to collaborative international efforts across disciplines and institutions. Large cohorts such as DABNI,[109] largely explained in the methodological section, and the Horizon 21 consortium, now comprising 10 centers (the Alzheimer-Down Unit, the LonDowns Consortium,[191] the Cambridge University, the Trinity College, the Geriatric outpatient clinic – Institut Jérôme Lejeune, Dichterbij – Center for Intellectual Disabilities, Vestfold Hospital Trust, University of Gothenburg, Karolinska Institute Hospital Huddinge and the Ludwig-Maximilians-Universität Münch) in Europe, or the Alzheimer's Biomarker Consortium Down Syndrome (ABC-DS),[157] the Lumind IDCS - Longitudinal Investigation for the Enhancement of Down Syndrome Research (LIFE-DSR)[192] in the United States, have studied the natural history of AD in DS through clinical and cognitive data, genetics and different biomarkers. In this line, the international non-profit scientific organizations Trisomy 21 Research Society (T21RS)[193] and the Alzheimer's Association Professional Interest Area in Down syndrome, have been created to facilitate and stimulate the interaction between researchers in the field, to establish common protocols for basic and translational research and for scientific dissemination. Consequently, the paradigm has changed and at the forefront of research priorities is the imperative to conduct trials against AD in this population. These consortiums are working to set the stage for conducting secondary preventive trials in AD and DS developing the appropriate tools and trial ready cohorts.

Alongside these consortiums, health institutions such as the NIH – National Institute on Aging (NIA) have funded clinical trials for adults with DS, most importantly the Alzheimer's Clinical Trial Consortium - Down Syndrome (ACTC-DS).[185] ACTC-DS is conducting now the first international project, the Trial Ready Cohort - Down syndrome (TRC-DS), which aims to enroll non-demented participants with DS worldwide in a longitudinal safety-run study using MRI, amyloid PET, cognitive testing, and fluid biomarkers in preparation for upcoming randomized placebo-controlled clinical trials for AD in DS. Another initiative worth mentioning is the INvestigation of Co-occurring conditions across the Lifespan to Understand Down syndromE (INCLUDE)[194] project, which was launched in June 2018 as a new trans-NIH research initiative on critical health and quality-of-life needs for individuals with DS, such as AD, autism, celiac disease, congenital heart disease, diabetes or COVID-19, which aim is to identify specific health gaps and prepare people with DS to participate in research for their own benefit.[194,195]

In short, there is unprecedented, coordinated efforts and funding to fight AD in the DS population. Research infrastructures and consortia have been built and the research in DS and AD has largely caught up with that in sporadic AD and ADAD. There are several trials soon to start in DS and a great challenge to bring to this population the new drugs that most likely will be approved soon. All this supports an optimistic view about the future.

5. Ethical considerations for research in intellectual disabilities

Individuals with ID have historically been fighting against discrimination and for their full inclusion in society by promoting respect from the most basic human rights, such as health, education, and freedom. In this sense, although adults with ID usually have substantial health disparities; they frequently experience decreased attention towards their health needs and preventive healthcare, as well as limited access to scientific breakthroughs. Due to the history of exploitation in this population, there are issues surrounding the ethical and legal implications for conducting research with individuals with DS. To overcome this prior abuse, specific laws have been developed. However, sometimes the line between protection and a new and subtle form of discrimination can be very thin. Law enforcement can easily remove these individuals' ability to make their own decisions and, for instance in the research context, might be denying them the opportunity of benefiting from health advances.

Despite the knowledge that AD is the main cause of death in people with DS,[133,134] this population has historically been excluded from AD clinical trials. This is in contradiction to the

United Nations Convention on the rights of persons with disabilities, which encourages participation of persons with disabilities in biomedical research.[196] Moreover, this limits the generalizability of the research results found in the general population. The recent active collaboration of people with DS in clinical research over the last decades has led to important scientific advances. There is now clear evidence to demonstrate that people with DS are ready and willing to be included in clinical trials.

There are, however, also risks and ethical challenges associated with research in vulnerable populations, and DS in particular. Researchers, caregivers, and specialized attention centers for individuals with ID will have to be aware and respectful of the specific and individual conditions of each person. Vulnerable participants in the context of research are those with a diminished ability to fully safeguard themselves.[197] It is thus highly important to respect participants' autonomy and self-determination in balance with our responsibility to protect them. Investigators must pay special attention to the three core research ethics principles: respect for personal autonomy, concern for welfare, and promotion of justice. Meeting this responsibility requires creativity and determination in resolving ethical dilemmas that contribute to exclusion from clinical trials.[198] The scientific community must make a great effort to give detailed and clear information by adapting the vocabulary and materials to facilitate the participants' adequate understanding of what it means to participate in research, the rights they have as a participant, and the related risks and benefits of enrolling in a research project. Moreover, vulnerable individuals should not be inappropriately included or automatically excluded from participation in research and participation should be based on appropriate inclusion or exclusion criteria consistent with the research question. In general, researchers should anticipate, as far as possible, the specific needs of participants. Finally, special ethical safeguards should be respected, and caregivers will have a prominent role in helping to make the decision to participate or not, as well as supporting individuals taking part in trials.[197]

Representation of individuals with ID in population health and research samples is crucial to develop a comprehensive understanding of their specific needs and develop adequate support and interventions.[199]

6. Limitations and future lines of research

In addition to the limitations detailed in each work, there are some common shortcomings in this thesis and gaps of knowledge that are important to explore.

First, there are no defined diagnostic criteria for symptomatic AD in DS, which limits the clinical management of patients and the scientific advances, compromising to a certain extent, for example, the study of the diagnostic and prognostic accuracy of cognitive tests and biomarkers. In this sense, in studies 1 and 2 we based the diagnosis on the neurologist's diagnosis alone, blinded to neuropsychological assessment. This decision was taken to avoid circularity in the analyses of the diagnostic performance of the neuropsychological tests, but it could have led to a misidentification of prodromal AD cases as asymptomatic, or to the inclusion of subjects with undetected medical, pharmacological, or psychiatric conditions interfering in cognition of the asymptomatic group. Of note, this might have resulted in an underestimate of the diagnostic performance on tests. Future studies should replicate our population norms, including larger samples and, ideally, biomarkers to correctly classify individuals in the different diagnostic categories in order to provide cutoffs for the different age ranges from the age of 35. It could also be interesting to describe normative data for longitudinal cognitive testing, or to develop diagnostic algorithms combining cognitive data with biomarkers.

Second, in neuropsychology, it is essential to establish the psychometric properties of the instruments, such as test-retest reliability and sensitivity to change a specific population. These psychometric properties have not been fully established for most of the instruments available today for adults with DS. Moreover, there is a need for new tests and measurement strategies to appropriately measure the different cognitive domains in adults with SD and to monitor cognitive changes in clinical trials. The measurement of cognitive decline in adults with DS and severe and profound ID remains a clinical unmet need. Most of the neuropsychological tools used to assess cognitive performance are not appropriate for severe and profound levels of ID, and these individuals are usually excluded from the cognitive analyses. For this reason, there is an urgent need to develop neuropsychological tests to assess AD-related cognitive decline in this part of the population. Digital cognitive biomarkers may offer new for individuals with ID in general and DS in particular. These measures will allow us to obtain more objective, ecologically valid, and continuous longitudinal data. The use of computerized testing provides the opportunity for strong standardization and automatic scoring, but this type of testing might be hindered by the relatively short attention span and the social nature of individuals with DS if the tasks are of less interest to them or too long.

Third, further longitudinal studies are needed to better understand the natural history of DS and its associated conditions, as well as the lifelong factors that may predispose to risk or resilience against AD. Although our studies are based on the largest cohort of individuals with

DS devoted to the study of the AD natural history, DABNI is still a novel cohort with a relatively short period of follow-up. Hence, inclusion and follow-up of the participants in the DABNI cohort is still active and, in the next few years, will be able to provide additional and more accurate results.

Fourth, individuals with DS have several co-occurring conditions that may influence the development and course of AD, as well as biomarkers and cognitive measures. Future studies should consider cardiovascular conditions, lifestyle, or educational level in this population to study its impact on AD. Accordingly, the impact of cognitive reserve in the age of onset and progression of AD in DS is of special interest to me; this issue is understudied in this population and needs further clarification.

Finally, as previously mentioned AD diagnostic criteria have recently incorporated the use of biomarkers for a more precise and individualized diagnosis. However, the relation between AD biomarkers and cognitive measures is still understudied. One way to identify the nature of cognitive changes associated with incipient Alzheimer's pathology is to characterize the cognitive performance of asymptomatic individuals in relation to the presence of AD biomarkers. We deeply explored the correlation between CSF-VAMP2 with ID level and cognitive performance along the AD continuum, but plasma NfL concentrations were superficially studied due to the heterogeneity in the cognitive evaluation protocols between the different sites. Future studies should incorporate multimodal AD-biomarkers to study their correlation with cognitive and clinical measures. Importantly, biomarkers cutoff points are also needed.

7. Final considerations

Due to the triplication of chromosome 21 causing DS this population has near full penetrance of symptomatic AD, with an estimated lifetime risk of dementia of the Alzheimer's type to be more than 95%.[114,131] Thus, recent clinical and biomarker evidence have led to the conceptualization of DS as a genetic form of this disease similar to ADAD.[115,200] The increase in life expectancy due to better treatment of other co-occurring conditions has put this association between DS and AD at the forefront of health policies in DS. Indeed, AD is the main cause of death in this population and imposes a limit to life expectancy more than 20 years below that in the general population.[133] However the clinical diagnosis of prodromal AD and AD dementia in DS remains a challenge mainly due to the premorbid ID associated with the

syndrome. Most importantly, there are no disease modifying treatments against AD in this population; clinical trials are an imperative. This thesis has contributed to these present and future endeavors by providing, for the first time, estimates of progression to symptomatic AD with age and longitudinal cognitive decline in different age ranges and the different clinical groups of the AD continuum. We have provided evidence to support the use of neuropsychological tests for symptomatic AD diagnosis, confronting a dogma in current diagnostic recommendations that emphasize longitudinal over cross-sectional assessments. Finally, it has contributed to the advance in the field of plasma AD-biomarkers experienced in the last 5 years in the DS population. In particular, the multicenter study assessing the longitudinal NfL changes might lay the foundation for its use as a theragnostic marker in clinical trials against AD.

Overall, our works have further demonstrated the inevitable progression to symptomatic AD from the fourth decade of life and the increasing risk with age. Immediate future works must define the clinical diagnostic criteria for AD in DS and population-based health plans to screen for symptomatic AD in adults with DS, such as the one that enabled DABNI and this thesis, should be generalized. Our works have also demonstrated that adults with DS can undertake the clinical, cognitive and biomarkers assessments for the duration of a disease-modifying clinical trial (typically 18 months to 3-5 years). Finally, we would like to emphasize that it might be easier to cure AD in DS than in the general population, and it is certainly, the best population in which to conduct preventive clinical trials because on the one hand, it is more homogeneous and predictable than sporadic AD and, on the other, DS is orders of magnitude more frequent than autosomal dominant AD.

CONCLUSIONS

The main conclusions of this thesis are:

1. The clinical progression to symptomatic Alzheimer's disease shows an exponential increase with age in asymptomatic adults with Down syndrome, while it is universal and independent of age in patients with prodromal Alzheimer's disease. Adapted cognitive tests are able to track the longitudinal AD-related cognitive decline in adults with DS and mild or moderate levels of intellectual disability.

2. It is possible to accurately diagnose prodromal Alzheimer's disease and Alzheimer's disease dementia using neuropsychological tests. The baseline cross-sectional cognitive evaluation provides higher accuracy than the one-year longitudinal cognitive change.

3. Plasma NfL is an excellent diagnostic and prognostic biomarker for Alzheimer's disease in Down syndrome. The longitudinal increases in all stages of the Alzheimer's disease continuum support its use as a theragnostic marker in clinical trials.

4. Synaptic biomarkers (and cerebrospinal VAMP-2 in particular) show promise as surrogate markers of Alzheimer's disease-related cognitive decline in Down syndrome that might aid in diagnosis, disease monitoring, and clinical trials.

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ANNEXES

Annex 1: Complementary work, Diagnosis of prodromal and Alzheimer's disease dementia in adults with Down syndrome using neuropsychological tests

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DIAGNOSTIC ASSESSMENT & PROGNOSIS

Diagnosis of prodromal and Alzheimer's disease dementia in adults with Down syndrome using neuropsychological tests

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Abstract

Introduction: We aimed to define prodromal Alzheimer's disease (AD) and AD dementia using normative neuropsychological data in a large population-based cohort of adults with Down syndrome (DS).

Methods: Cross-sectional study. DS participants were classified into asymptomatic, prodromal AD and AD dementia, based on neurologist's judgment blinded to neuropsychological data (Cambridge Cognitive Examination for Older Adults with Down's syndrome [CAMCOG-DS] and modified Cued Recall Test [mCRT]). We compared the cutoffs derived from the normative data in young adults with DS to those from receiver-operating characteristic curve (ROC) analysis.

Results: Diagnostic performance of the CAMCOG-DS and modified Cued Recall Test (mCRT) in subjects with mild and moderate levels of intellectual disability (ID) was high, both for diagnosing prodromal AD and AD dementia (area under the curve [AUC] 0.73–0.83 and 0.90–1, respectively). The cutoffs derived from the normative data were similar to those derived from the ROC analyses.

Discussion: Diagnosing prodromal AD and AD dementia in DS with mild and moderate ID using population norms for neuropsychological tests is possible with high diagnostic accuracy.

KEYWORDS
Down syndrome, Alzheimer's disease, dementia, cognitive testing, assessment, CAMCOG-DS, Cued Recall Test, normative data

1 | INTRODUCTION

Due to advances in medical care, life expectancy has increased significantly in people with Down syndrome (DS), now exceeding 60 years of age.[1,2] As a consequence, individuals with DS are now experiencing a high incidence of age-associated health problems,[3] especially Alzheimer's disease (AD) dementia. Pathological studies show that by the age of 40 years, virtually all individuals with DS have AD neuropathology,[4] and longitudinal studies show that the cumulative incidence of dementia in adults with DS is in excess of 90% by age 65.[1,5] Symptomatic AD increases exponentially with age, with a mean age at dementia onset of between 53.7 and 55.8 years,[6,7] and approximately 50% of cases of dementia are being diagnosed in the sixth decade of life.[6,7] This association between DS and AD is explained mainly by the presence of an extra copy of the amyloid precursor protein (*APP*) gene, located on chromosome 21.[8]

The diagnosis of prodromal AD and AD dementia in DS is a major challenge. Early symptoms of AD can be mistaken as part of the lifelong intellectual disability (ID), or they may be overlooked or misdiagnosed. In the general population, the diagnosis of mild cognitive impairment requires a change in cognition reported by the patient and/or caregiver, and is based on cognitive performance on neuropsychological tests relative to population norms, and dementia is diagnosed when cognitive decline affects the activities of daily living. In people with DS, the variable degree of ID problematizes these definitions. Furthermore, most test batteries commonly used in the general population are of limited use in DS, as many individuals score at floor and noncompletion rates are high.[9,10]

Adapted tests have been developed to detect cognitive decline in DS, such as the Down Syndrome Mental Status Examination (DSMSE), the Test for Severe Impairment (TSI), the Cambridge Cognitive Examination for Older Adults with Down's Syndrome (CAMCOG-DS), and the Arizona Cognitive Test Battery (ACTB) or the modified Cued Recall Test (mCRT).[3,9,11-19] However, unlike in the general population, in adults with DS we are lacking normative data due to the large sample sizes needed to account for the different levels of ID and its associated variability in cognitive abilities. Furthermore, although the diagnostic performance of several adapted tests has been assessed, most of the studies have used small sample sizes and did not take into account the level of ID. Therefore, the diagnosis of prodromal AD or AD dementia using neuropsychological tests in this population at the cross-sectional level is difficult and cannot be made reliably on the basis of neuropsychological tests using population norms.[20]

Taking advantage of the Down Alzheimer Barcelona Neuroimaging initiative (DABNI), a large population-based cohort of adults with DS, the purpose of our study was to define population norms stratified by level of ID for the CAMCOG-DS and the mCRT, a cognitive battery and an episodic memory test widely used in DS, and to assess their performance to diagnose prodromal AD and AD dementia in adults with DS.

2 | METHODS

2.1 | Participants

Single-center cross-sectional study. Adults with DS were recruited from February 1, 2013 to December 31, 2018 at the Alzheimer-Down Unit from the Catalan Down Syndrome Foundation and Hospital de la Santa Creu i Sant Pau, in Barcelona, Spain. The Alzheimer-Down Unit leads a population-based health plan for adults with DS, which includes yearly neurological and neuropsychological assessments. All adults (≥18 years) with DS were eligible, irrespective of sex or level of ID. Patients showing any psychiatric or medical disorder that could affect cognition and/or functionality were excluded, as well as those with incomplete neuropsychological examinations (flow chart in Figure 1).

The study was approved by the Sant Pau Ethics Committee following the standards for medical research in humans recommended by the Declaration of Helsinki and reported to the Minister of Justice according to the Spanish law for research in people with intellectual disabilities. All participants or their legally authorized representative gave written informed consent before enrollment.

2.2 | Neurological assessment

The study procedures included a complete neurological examination with the participant and his/her main caregiver. The neurologist performed a physical exam, a structured medical history based on the DS-Connect questionnaire,[21] a neurological exam, and a semi-structured health questionnaire (Cambridge Examination for Mental Disorders of Older People with Down's Syndrome and others with intellectual disabilities [CAMDEX-DS]) with the caregiver. The CAMDEX-DS is a diagnostic tool based upon CAMDEX-R and modified for the detection of dementia in people with ID.[20] It is also adapted and validated for the Spanish population with ID.[22]

2.3 | Neuropsychological assessment

The neuropsychological test battery for detecting dementia included the Cambridge Cognitive Examination for Older Adults with Down's

Syndrome (CAMCOG-DS) Spanish version[22] and the mCRT,[18] both directly administered to the patient. It also included the Dementia Questionnaire for People with Learning Disabilities (DLD), an informant-based questionnaire to assess cognitive and functional decline due to dementia.[23]

The CAMCOG-DS has a maximum score of 109 points and comprises subscales for the following cognitive domains: orientation, language, memory, attention, praxis, abstract thinking, and perception.

The mCRT is an adapted test used to assess episodic memory in people with ID. Participants are asked to memorize 12 stimuli presented on three, 4-item cards. The test consists of three trials of free and cued recall performed immediately after the learning phase to compute a free recall score and a total score (free recall + cued recall). For the present study we used the total score (maximum score of 36), as it was shown to be more sensitive to detect memory decline in DS.[19]

2.4 | Level of ID

The level of ID was categorized according to the *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition* (DSM-V) as mild, moderate, severe, or profound, and was based on caregivers' reports of the individuals' best-ever level of functioning. The Kauffman Brief Intelligence Test (K-BIT) was also included to assess pre-morbid intelligence level.

2.5 | Diagnostic categories

In our center, participants with DS are initially clinically classified by neurologists and neuropsychologists in a consensus meeting after independent visits into the following diagnostic categories: (1) asymptomatic (aDS), when there is no clinical or neuropsychological suspicion of AD; (2) prodromal AD (pDS), when there is a suspicion of AD, but symptoms do not fulfill criteria for dementia; (3) AD dementia (dDS) in those subjects with DS with full blown dementia; and (4) uncertain, including those patients with medical, pharmacological, or psychiatric conditions significantly interfering in cognition and/or functional level. As mentioned previously, patients with an uncertain diagnosis were excluded from the analysis. It is important to note that to avoid circularity, in this study we used the initial neurologist's diagnosis blinded to neuropsychological assessment.

2.6 | Statistical analysis

All statistical analyses were done in R version 3.4.3. First, to assess the applicability of the tests, we analyzed the completion rates by level of ID and clinical status. Second, we compared the neuropsychological performance between clinical groups stratifying by the level of ID using an analysis of variance (ANOVA) for normal variables or Kruskal-Wallis for non-normal variables. Third, we studied the relationship of the different neuropsychological tests with age. For this purpose, we fitted a local regression model stratified by level of ID.[7] Finally, to define cutoffs for the neuropsychological tests, we used two different approaches. The first approach consisted in defining the scores at percentile ranks of 1st, 5th, and 10th in the young (age $\leq$ 35) asymptomatic DS participants in mild and moderate ID separately.[24] This decision was based on the fact that all subjects with DS show the characteristic neuropathological signs of AD by the fourth decade of life. Subjects >35 years were expected to have AD neuropathology and thus could have undergone cognitive deterioration. The second approach consisted of using the receiver-operating characteristic (ROC) curve analyses. The optimal cut-point was determined using the Index of Union (IU) method, which is defined as the value whose sensitivity and specificity are the closest to the value of the area under the ROC curve and the absolute value of the difference between the sensitivity and specificity values is minimum. The criteria for optimality can change according to the aim of the study. However, as a general rule, minimizing the total misclassification rates is a good approach. With the IU method, since the difference between sensitivity and specificity values is minimum, this condition is met most of the time.[25]

RESEARCH IN CONTEXT

1. Systematic review: Literature was reviewed through PubMed and meeting abstracts. Due to the variability of intellectual functioning in people with Down syndrome (DS), there are no accepted population-based neuropsychological normative data and very few studies investigating the diagnostic performance of cognitive tests for diagnosing prodromal Alzheimer's disease (AD) and AD dementia in this population.
2. Interpretation: In a large population-based cohort of adults with DS with mild and moderate levels of intellectual disability (ID), neuropsychological normative data for Cambridge Cognitive Examination for Older Adults with Down's Syndrome (CAMCOG-DS) and modified Cued Recall Test (mCRT) were provided. We show that a diagnosis of prodromal AD and AD dementia can be done with high diagnostic accuracy.
3. Future directions: Our results support the use of the CAMCOG-DS and mCRT as screening tools for the diagnosis of AD in people with DS with mild and moderate ID. Our cutoffs could be used for screening purposes in both clinical practice and research settings.

3 | RESULTS

A total of 567 adults with DS were eligible. Figure 1 shows the study flow chart and the reasons for exclusion, mainly due to an uncertain diagnosis or incomplete neuropsychological assessment. The initial samples for the CAMCOG-DS and the mCRT were composed of

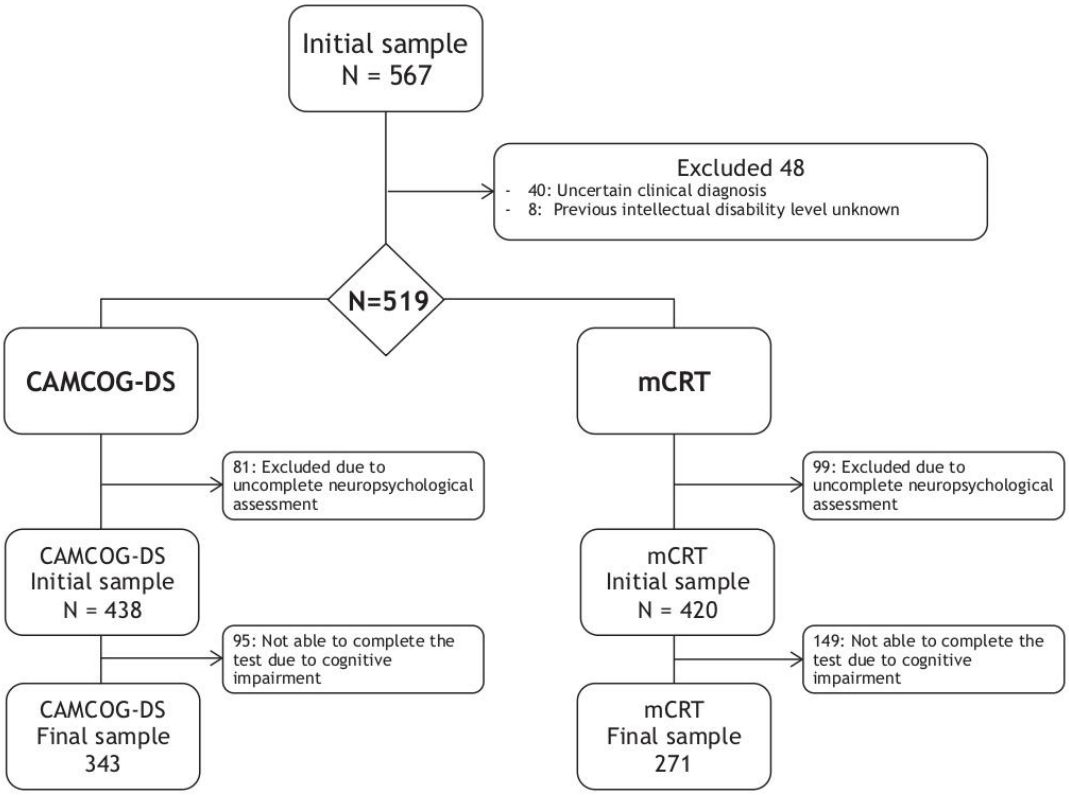

FIGURE 1 Study flow chart. mCRT, modified Cued Recall Test; CAMCOG-DS, Cambridge Cognitive Examination for Older Adults with Down's Syndrome

438 and 420 subjects, respectively. Of note, age and level of ID differed between those subjects included and excluded from the study. Subjects who did not attend the neuropsychological visit were older and had more severe levels of ID ($P < .05$). Demographic and clinical characteristics of participants from the CAMCOG-DS and mCRT initial sample are shown in the Appendix.

As expected, participants with prodromal AD and AD dementia were older and had worse scores than asymptomatic subjects on the DLD ($P < .001$). There were no differences in the number of men and women between the groups. There was a higher proportion of subjects with severe/profound ID in the group with AD dementia.

3.1 | Completion rates by level of ID and clinical diagnosis

Figure 2 shows the CAMCOG-DS and mCRT completion rates by level of ID and clinical diagnosis. The most common reasons for not completing the test were not understanding the task and/or test instructions and/or severe attentional difficulties.

Overall, of the 438 subjects from the initial CAMCOG-DS sample, 343 (78.3%) subjects were able to complete the test. Completion rates were lower for the mCRT, where 271 of 420 subjects (64.5%) could complete the task. All subjects with mild ID, regardless of clinical diagnosis, completed the tests, as did the majority of asymptomatic and prodromal AD subjects with moderate levels of ID. Completion rates were lower in those with pDS and dDS than in aDS. None of the subjects with profound ID and only a few subjects with severe ID were able to complete the tests. Therefore, subjects with severe and profound levels of ID were excluded from the subsequent analyses (normative data and ROC analysis).

3.2 | Cognitive performance with aging and along the AD continuum by level of ID

Median scores and interquartile range for the cognitive and functional tests in those who completed the tests are presented in Table 1. Participants with mild ID obtained higher scores on the CAMCOG-DS than subjects with moderate ID in the whole AD continuum (median scores in aDS: 89 vs 75, $P < .0001$; pDS: 79 vs 60.5, $P = .0013$; dDS: 76.5 vs 55,

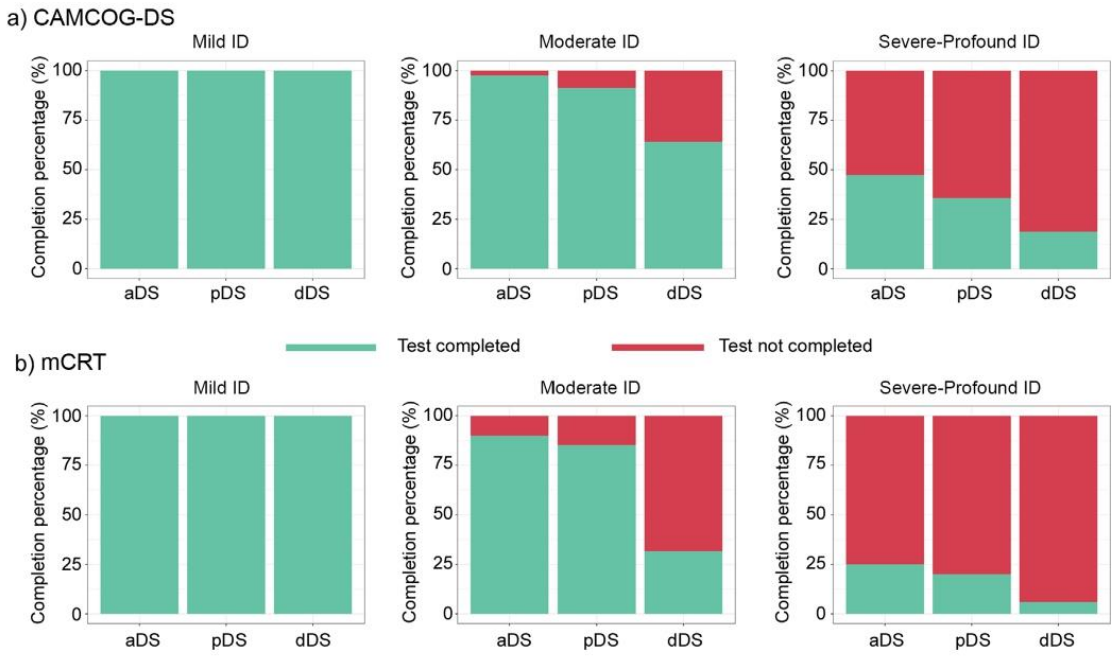

FIGURE 2 Completion rates for the CAMCOG-DS and mCRT by level of intellectual disability and by diagnostic group. mCRT, modified Cued Recall Test; CAMCOG-DS, Cambridge Cognitive Examination for Older Adults with Down's Syndrome; ID, intellectual disability; aDS, asymptomatic Down syndrome; pDS, prodromal Down syndrome; dDS, dementia Down syndrome

$P = .021$) (Figure 3). Total scores on the mCRT were significantly different between subjects with mild and moderate ID in aDS (median 36 vs 35, respectively, $P = .0002$), but not in pDS (28 vs 27, respectively, $P = .92$) and dDS (21 vs 15, respectively, $P = .37$) participants.

We found no differences between male and female participants in the cognitive scores. There was an age effect on CAMCOG-DS and mCRT scores, both in the whole cohort and in aDS individuals (Figure 4). There was a progressive decline on both the CAMCOG-DS and mCRT after age 40, and especially for the subgroup of participants with moderate ID.

3.3 | Normative data for the CAMCOG-DS and mCRT in asymptomatic DS individuals

To exclude preclinical AD, we derived normative data in the younger subjects (≤35 years: 107 subjects for the CAMCOG-DS and 89 subjects for the mCRT). Normative data were generated in aDS individuals in mild and moderate ID separately. Scores corresponding to the 1st, 5th, and 10th percentiles were used to define pathological performances (see Appendix for further details).

For the CAMCOG-DS, cut-points corresponding to the 1st, 5th, and 10th percentiles for subjects with mild ID were, respectively, 77, 78, and 80. In subjects with moderate ID, cut-points corresponding to these percentiles were as follows: 48, 53, and 59, respectively.

For the mCRT, cut-points for the participants with mild ID were 34 for the 1st percentile, and 35 for the 5th and 10th percentiles. For those subjects with moderate ID, these percentiles corresponded to a score of 30, 32, and 33, respectively.

3.4 | Diagnostic performance

To assess the diagnostic performance to detect prodromal AD and AD dementia we performed ROC curve analyses. All subjects with DS with mild ID and a very high proportion of subjects with moderate ID were able to complete the tests. However, a subset of participants with moderate ID and symptomatic AD had difficulties understanding the instructions of the CAMCOG-DS and/or the mCRT due to cognitive difficulties, and thus received a score of "0" (represented in purple in Figure 3). These subjects were excluded from these analyses due to concerns about construct validity.

Figure 5 shows the ROC analyses. The AUC for CAMCOG-DS for the aDSversus pDS comparison in mild ID was 0.81 (95% CI 0.55–1), and the optimal cutoff point was 82, with a sensitivity of 80% and a specificity of 80.5%. Comparing aDS versus dDS, we obtained an AUC

TABLE 1 Demographic characteristics, median scores, and interquartile range (IQR) for the cognitive and functional tests of the participants who completed the CAMCOG-DS and the mCRT

CAMCOG-DS subgroup				
	aDS	pDS	dDS	TOTAL
N	277	31	35	343
Sex (F/M)	132/145	18/13	19/16	168/174
Age (median years [IQR])	37 [15.0]	51 [4.5]	53 [8.5]	41 [18.5]
ID (Mild/Moderate/Severe+Profound)	82/159/36	5/21/5	4/25/6	91/205/47
CAMCOG-DS (median [IQR])	78 [25.0]	58 [24.5]	45 [26.5]	73 [30.0]
DLD (median [IQR])	12 [14.0]	21[19.5]	43 [17.8]	14 [17.0]
mCRT subgroup				
N	220	31	20	271
Sex (F/M)	101/119	17/14	10/10	128/143
Age (median years [IQR])	36 [15.0]	51 [5.0]	54 [7.0]	39 [18.0]
ID (Mild/Moderate/Severe+Profound)	75/125/20	5/23/3	5/13/2	85/161/25
mCRT (median [IQR])	36.0 [2.0]	27.0 [16.0]	15.5 [11.3]	35.0 [4.0]
DLD (median [IQR])	11 [12.0]	17.5 [13.5]	35 [24.0]	13 [13.0]

aDS, asymptomatic Down syndrome; CAMCOG-DS, Cambridge Cognitive Examination for Older Adults with Down's Syndrome; dDS, dementia Down syndrome; DLD, Dementia Questionnaire for People with Learning Disabilities; F, female; ID, intellectual disability; IQR, interquartile range; M, male; mCRT, Modified Cued Recall Test; pDS, prodromal Down syndrome.

of 0.91 (95% CI 0.78–1) and an optimal cutoff point of 80, with a sensitivity of 75% and a specificity of 87.8%. In the subgroup of participants with moderate ID, the AUC in the aDS versus pDS comparison was 0.73 (95% CI 0.63–0.83), with a cutoff point of 64, a sensitivity of 66.7%, and a specificity of 72.3%. When comparing aDS versus dDS, we found an AUC of 0.90 (95% CI 0.83–0.97) and a cutoff point of 56, with a sensitivity of 84% and a specificity of 84.3%.

In the ROC analysis for the mCRT scores (Figure 5), the AUC for the comparisons between aDS and pDS with mild ID was 0.79 (95% CI 0.38–1), with a cutoff point of 35, a sensitivity of 66.7%, and a specificity of 73.3%. Comparing aDS versus dDS, we obtained an AUC of 1 (95% CI 1–1), with a cutoff point of 29, a sensitivity of 100%, and a specificity of 100%. In the subgroup of participants with moderate ID, we obtained an AUC for the comparison between aDS and pDS of 0.83 (95% CI 0.73–0.94) and a cutoff point of 33, with a sensitivity of 78.3% and a specificity of 79.2%. When comparing aDS versus dDS, we found an AUC of 0.97 (95% CI 0.93–1) and a cutoff point of 28, with a sensitivity of 92.3% and a specificity of 94.4%.

Normative values corresponding to the 1st, 5th, and 10th percentiles obtained in young aDS with DS were comparable to those cutpoints obtained by ROC analysis for the detection of symptomatic AD for both CAMCOG-DS and mCRT (Appendix, Table S2).

4 | DISCUSSION

This is the first study to show the applicability of the mCRT and the CAMCOG-DS in a large population-based cohort stratified by level of ID, and also the first to show the diagnostic performance to detect prodromal AD and AD dementia. We showed that it is possible to diagnose symptomatic AD using population norms in people with DS through the administration of neuropsychological tests in adults with mild and moderate ID. The cutoff points derived from the normative data were in agreement with the thresholds in the ROC analyses.

Completion rates for the CAMCOG-DS and mCRT greatly varied by the severity of ID and along the AD continuum, with the mCRT showing lower completion rates. This was an expected result, as these tests were designed to assess cognition in mild and moderate ID. Virtually all aDS with mild and moderate ID completed the tests compared to less than 50% of subjects with severe ID and none of the subjects with profound ID. Similarly, symptomatic AD also affected completion rates. Although most pDS with mild or moderate levels of ID could complete the tests, completion rates decreased in dDS with moderate ID. These results show that assessing cognition in mild to moderate ID is feasible along the AD continuum, but that other instruments with lower floor effects should be used in severe ID.

The level of ID had a greater impact on CAMCOG-DS scores than on mCRT scores. Subjects with mild ID obtained significantly higher scores on the CAMCOG-DS than subjects within the moderate range. The mCRT, however, was less sensitive. As in our previous report on the natural history of AD in DS, cognitive decline was detectable after age 40, especially in moderate ID,[7] in agreement with previous studies.[26-28] The median age at diagnosis in our aforementioned study was 50.2 years for pDS and 53.7 years for dDS.[7] Therefore, cognitive decline is detectable cross-sectionally 10 years before symptomatic AD and occurs in parallel to hippocampal atrophy,[7] after amyloid and tau biomarkers become abnormal. These results reinforce that most of the decline associated with aging in DS is AD related. It is important to consider that all subjects with DS show the characteristic neuropathological signs of AD by the fourth decade of life.[4,29,30] For this reason, in

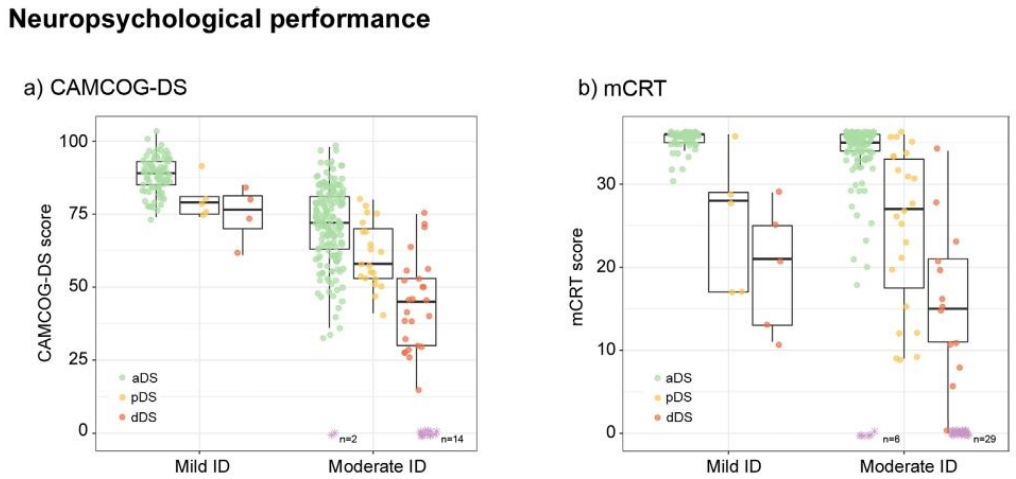

FIGURE 3 Neuropsychological performance of Down syndrome by clinical groups according to level of intellectual disability. ID, intellectual disability; aDS, asymptomatic Down syndrome; pDS, prodromal Down syndrome; dDS, dementia Down syndrome; mCRT, modified Cued Recall Test. CAMCOG-DS, Cambridge Cognitive Examination for Older Adults with Down's Syndrome. A subset of participants with moderate ID had difficulties understanding the instructions of the CAMCOG-DS and/or the mCRT due to cognitive difficulties, and thus received a score of "0" on these tasks (represented in purple at the bottom of the figure)

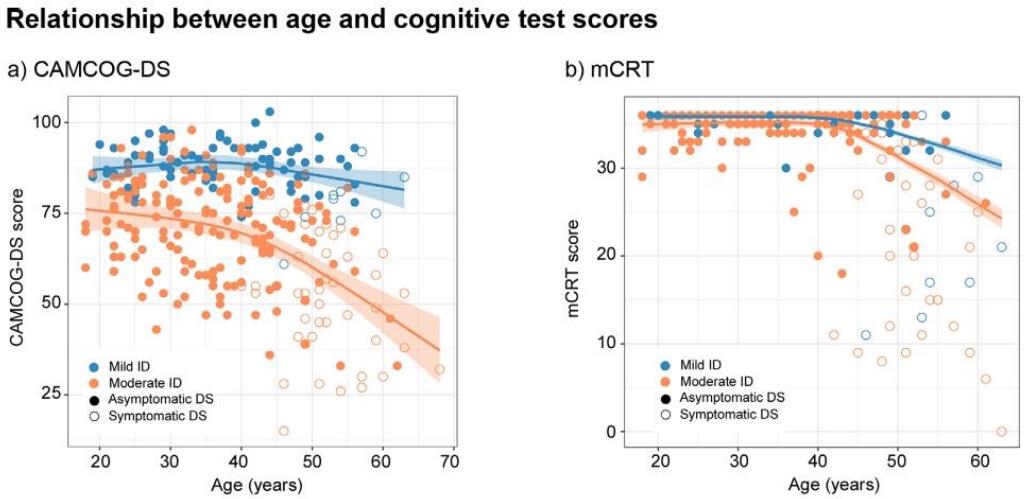

FIGURE 4 Relationship between age and cognitive scores in subjects with mild and moderate ID for the whole cohort. Footnote: mCRT, modified Cued Recall Test; CAMCOG-DS, Cambridge Cognitive Examination for Older Adults with Down's Syndrome; ID, intellectual disability; DS, Down syndrome

order to derive the population norms, we chose the younger individuals (≤35 years).

There was a clear decrease in both the CAMCOG-DS and mCRT values along the AD continuum. ROC analyses showed good diagnostic performance for the CAMCOG-DS and the mCRT. The cutoff score for the CAMCOG-DS derived from the ROC analyses achieved high sensitivity and specificity to diagnose AD dementia in both mild and moderate ID. Prodromal AD could also be diagnosed with good accuracy in mild ID, but not in moderate levels of ID. The mCRT showed higher diagnostic performance than the CAMCOG-DS. To the best of our knowledge, only one group has reported on CAMCOG-DS cutoff scores for the diagnoses of AD dementia in people with DS.[22] In the validation study of the CAMDEX-DS in Spain, Esteba et al. found a cutoff score of 68 in mild ID and 52 in moderate ID,[22] lower than in our study

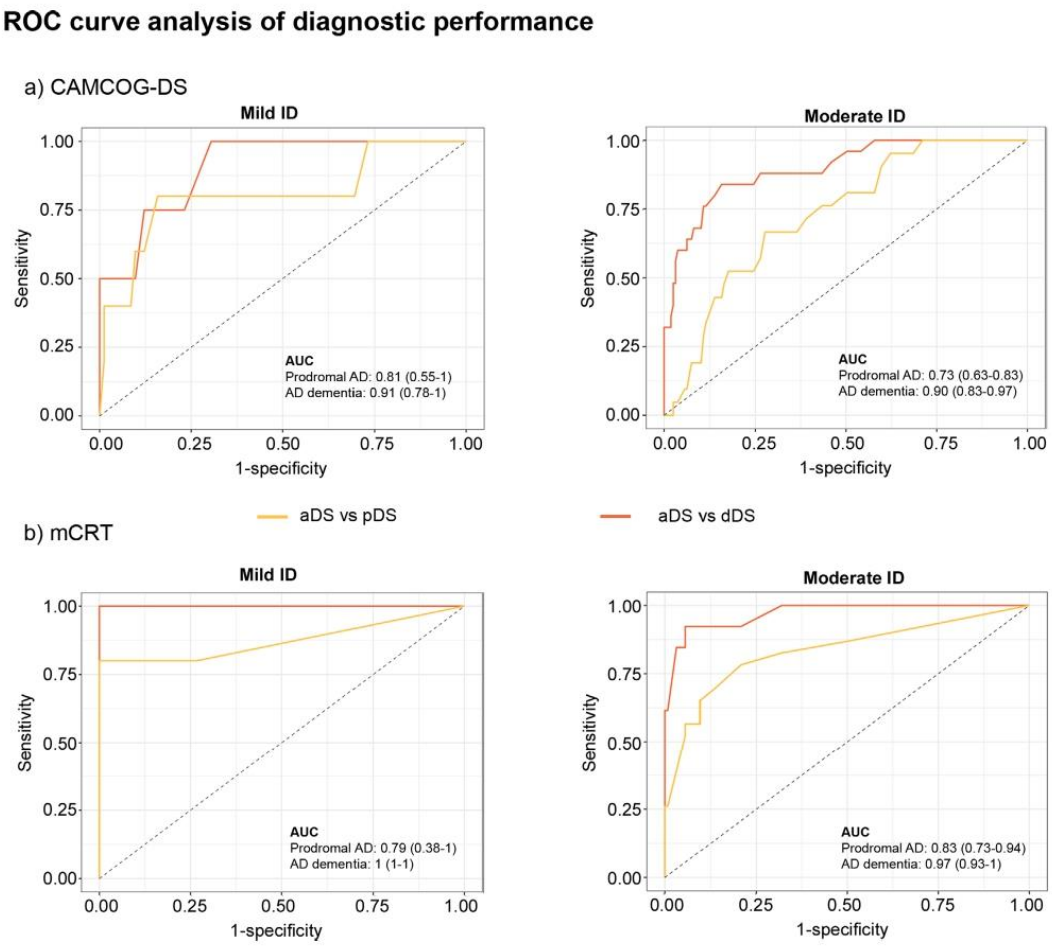

FIGURE 5 ROC curve analysis of diagnostic performance of CAMCOG-DS total score (a) and the mCRT total immediate recall score (b) for Down syndrome clinical groups (prodromal DS and dementia DS). pDS, prodromal Down syndrome; dDS, dementia Down syndrome; mCRT, modified Cued Recall Test; CAMCOG-DS, Cambridge Cognitive Examination for Older Adults with Down's Syndrome; ID, intellectual disability

(we obtained cutoff scores of 80 and 56, respectively). The inclusion of subjects with less cognitive decline in our study, which is based on a population health plan with active screening for AD, might explain the discrepancies. Cued recall tasks[31] are commonly used in the general population to differentiate between memory decline related to aging and AD-related memory deficits.[32,33] aDS individuals often performed at ceiling on the total score of the test. It is notable that this test was less sensitive to the level of ID than the CAMCOG-DS, and the cut-offs were very similar in the mild and moderate levels of ID. This lower variability associated to the level of ID facilitates diagnosis. Cognitive functions have been shown to decline sequentially in people with DS, with different cognitive domains being affected at different stages of the disease.[15,34,35] A longitudinal study conducted by Krinsky-McHale et al.[36] found that participants with DS with early stage AD dementia showed severely diminished episodic memory. Again, our cutoff scores of 29 and 28 (for the mild and moderate level of ID, respectively), derived from the ROC analyses, are higher than that reported by Devenny et al.,[18] who proposed a provisional cutoff score of 23 to identify dementia due to AD in DS populations.[18] Similar to the CAMCOG-DS results, these differences could be explained by the inclusion in our sample of subjects with earlier stages of AD, and thus with less impairment in memory functions.

We provide several thresholds for the normative data for the CAMCOG-DS and the mCRT obtained in younger asymptomatic subjects with DS (aged $\leq$ 35 years) with mild and moderate levels of ID (1st, 5th, and 10th percentiles). These cutoffs were in agreement with those established for the diagnosis of prodromal AD and AD dementia using ROC analyses (with the exception of the mCRT cutoffs derived in the ROC analyses for AD dementia). CAMCOG-DS scores below 80 in subjects with mild ID and below 59 in those with moderate ID were

indicative of a pathological performance on this test (corresponding to the 10th percentile). In the case of the mCRT, total immediate scores below 35 and 33 for those subjects with mild and moderate ID, respectively, could be considered at risk for symptomatic AD (both prodromal AD and AD dementia). Therefore, the 10th percentile cutoffs could be used for screening purposes in clinical practice. However, to diagnose AD dementia, more stringent thresholds should be used, especially for the CRT.

Taking into account the sensitivity and specificity obtained in both the normative data and from the ROC curve analysis, the cutoff points that we would recommend for the diagnosis of AD dementia in people with DS would be the following: In mild ID, CAMCOG-DS scores of 80 and mCRT scores of 29; in moderate ID, CAMCOG-DS scores of 56 and mCRT scores of 28.

The main strengths of this study are the large sample size and the fact that it comes from a large population-based cohort of adults with DS representative of the DS population in Catalonia. This enabled us to establish robust population-based norms in young aDS stratified by the level of ID. Another strength is that we could compare the thresholds derived from this approach to those of the ROC analyses, showing that the thresholds were comparable and yielded good diagnostic performance. This study also has some limitations. First, it is based on cross-sectional data. Second, it is a single-center study, and thus needs to be replicated in other cohorts and populations to confirm the generalizability of our results. Third, despite the large overall sample size, the number of subjects with prodromal AD and AD dementia with mild ID was reduced. For this reason we did not perform an internal cross-validation in the ROC approach. Fourth, to avoid circularity, we used the neurologist's initial diagnosis, blinded to neuropsychological assessment, and this could have led to a misidentification of prodromal AD cases as asymptomatic or to the inclusion in the asymptomatic group of subjects with medical, pharmacological, or psychiatric conditions that could have effects on cognition not detected by the neurologist. Of note this might have underestimated the diagnostic performance of the tests. Further studies with longitudinal follow-up and/or biomarkers would allow for more reliable diagnosis.[37]

In summary, our study shows that, similar to the general population, a diagnosis of prodromal AD and AD dementia can be done reliably in adults with DS based on the observation of low levels of cognitive test performance relative to population norms when stratifying by the level of ID.

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CONFLICTS OF INTEREST

Dr. Fortea has received compensation for consultancies to Novartis and AC Immune. The remaining authors have no conflicts of interest to declare that are relevant for this article.

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SUPPORTING INFORMATION

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

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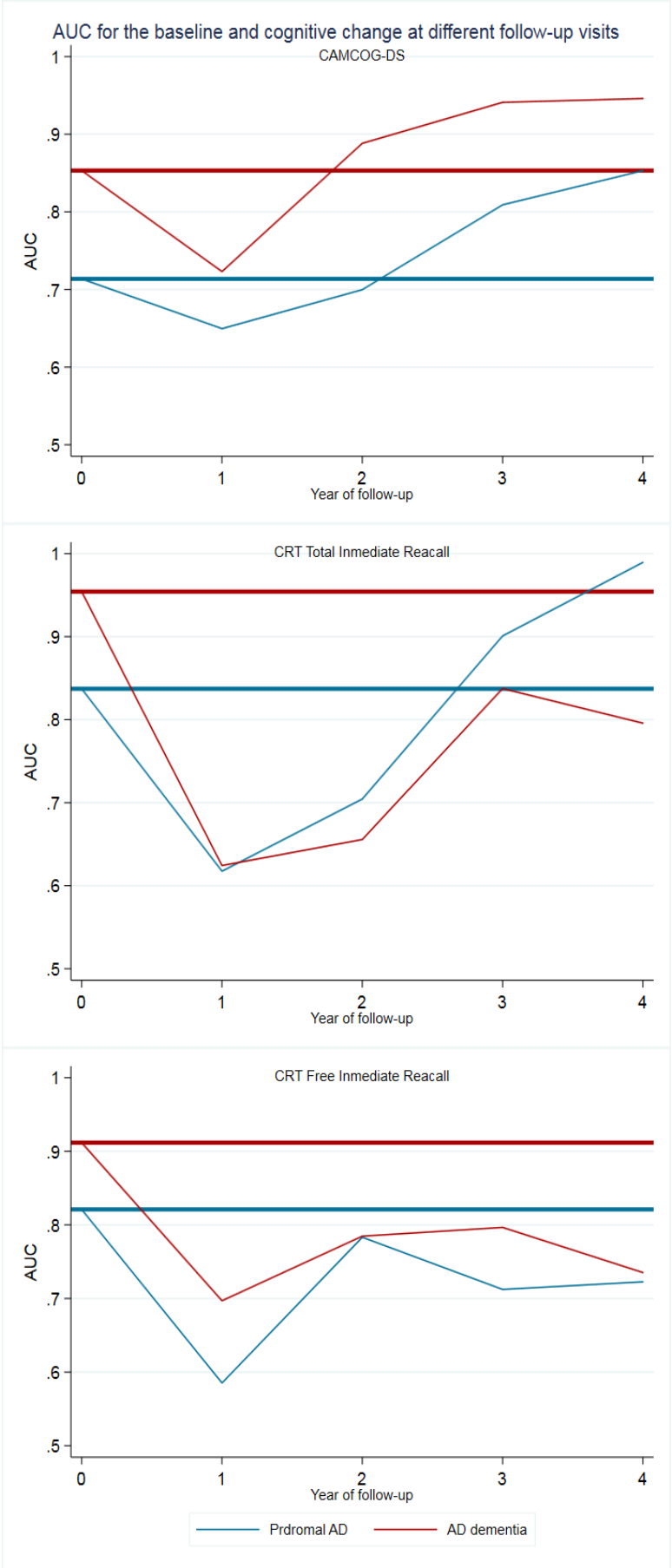
Annex 2: Supplementary material from the second work of this thesis "Cross-sectional versus longitudinal cognitive assessments for the diagnosis of symptomatic Alzheimer's disease in adults with Down syndrome"

Supplementary Table 1 Cognitive data by clinical diagnosis at baseline and cognitive change at each time point of follow-up

Whole sample, n=589	**Asymptomatic, n=440**	**Prodromal, n=63**	**Dementia= 86**
CAMCOG-DS (n, mean (sd))			
mild – Baseline,	149, 87.29 (7.23)	12, 78.58 (6.86)	11, 59.27 (19.66)
1-year ΔCog	95, 1.55 (4.89)	17, -3.18 (6.40)	24, -5.67 (7.12)
2-year ΔCog	76, 2.09 (5.25)	6, -0.33 (3.50)	18, -8.61 (10.28)
3-year ΔCog	69, 2.16 (5.19)	3, -2.67 (3.21)	9, -17.33 (12.62)
4-year ΔCog	54, 2.63 (4.90)	2, -2.5 (3.54)	7, -26.57 (25.19)
moderate - Baseline	277, 67.75 (13.43)	46, 58.48 (10.12)	71, 44.42 (13.74)
1-year ΔCog	154, 1.42 (6.10)	72, -4.94 (9.56)	44, -1.5 (6.05)
2-year ΔCog	143, 1.92 (6.03)	18, -3.28 (6.88)	30, -12.9 (10.60)
3-year ΔCog	119, 1.08 (6.88)	6, -10.33 (9.79)	14, -20.57 (16.60)
4-year ΔCog	89, 0.64 (7.08)	2, -15 (0)	11, -24.73 (17.42)
mCRT TIR (n, mean (sd))			
mild - Baseline	141, 35.37 (1.44)	10, 27.5 (7.56)	11, 17.09 (9.80)
1-year ΔCog	86, 0.17 (1.25)	15, -1.27 (3.61)	19, -1.05 (5.87)
2-year ΔCog	71, 0.35 (1.20)	6, -2.17 (2.99)	11, -4.45 (7.59)
3-year ΔCog	66, -0.18 (1.62)	3, -5.33 (4.04)	7, -7.14 (9.49)
4-year ΔCog	50, -0.06 (0.79)	2, -3 (1.41)	2, 2.5 (6.36)
moderate - Baseline	242, 33.66 (3.84)	49, 25.16 (8.48)	40, 18.43 (8.71)
1-year ΔCog	128, 0.64 (2.41)	38, -1.13 (6.38)	33, -1.58 (6.65)
2-year ΔCog	114, 0.73 (2.50)	14, -3.71 (5.86)	12, -0.42 (7.68)
3-year ΔCog	98, 0.34 (2.76)	5, -3.8 (3.90)	6, -5.5 (3.73)
4-year ΔCog	69, 0.72 (2.05)	2, -10.5 (3.54)	3, -7.33 (1.15)
mCRT FIR (n, mean (sd))			
mild - Baseline	141, 20.85 (4.46)	10, 13.3 (3.97)	11, 7.82 (5.72)
1-year ΔCog	86, 0.98 (4.02)	15, -0.87 (4.60)	18, -2.39 (3.05)
2-year ΔCog	71, 1.84 (3.70)	6, 0.5 (4.42)	11, -2.55 (4.34)
3-year ΔCog	65, 1.42 (3.85)	3, -1.33 (9.50)	7, -4.29 (5.88)
4-year ΔCog	50, 1.76 (3.62)	2, 1.5 (3.54)	2, 2.5 (2.12)
moderate	242, 16.96 (5.69)	49, 10.08 (5.31)	40, 7.85 (4.58)
1-year ΔCog	128, 0.56 (4.60)	38, -0.68 (4.89)	33, -1.94 (4.23)
2-year ΔCog	114, 1.48 (4.60)	14, -5.79 (4.44)	12, -2.33 (2.74)
3-year ΔCog	98, 0.67 (5.88)	5, -4.2 4.82)	6, -5.5 (3.83)
4-year ΔCog	69, 0.80 (4.92)	2, -7 (0)	3, -6.67 (1.53)

Supplementary figure 1: AUC for baseline and cognitive change at different follow-up visits for the CAMCOG-DS, the mCRT Total Immediate Recall and the mCRT Free Immediate Recall in the whole sample (mild and moderate ID together).



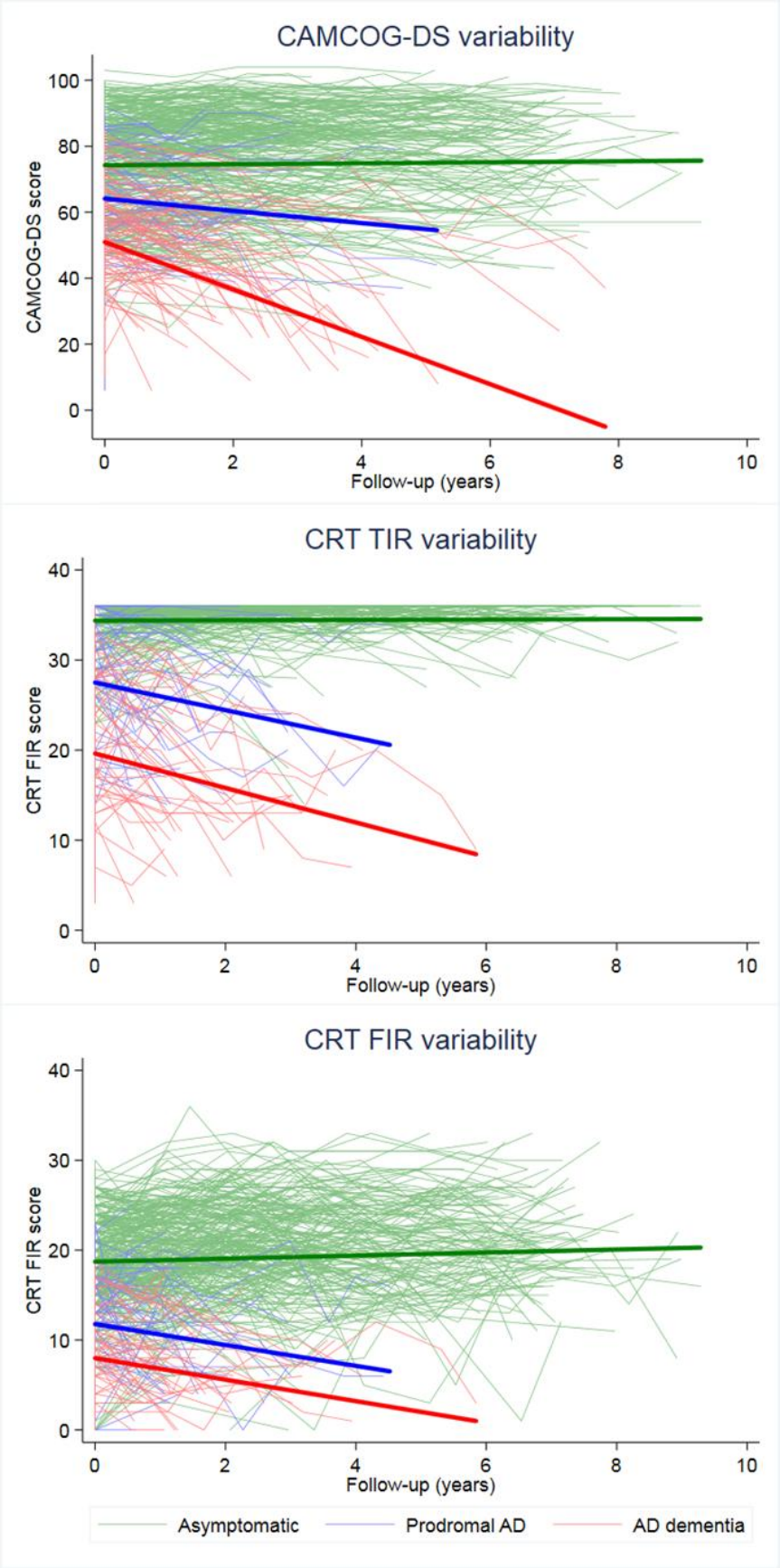
Supplementary table 2: AUC for longitudinal cognitive change with respect to baseline in mild and moderate ID groups and in the whole sample.

	AUC Raw difference with respect to baseline - Mild ID					
	CAMCOG-DS		CRT TIR		CRT FIR	
	pDS	dDS	pDS	dDS	pDS	dDS
Baseline	0,7579	0,9093	0,808	0,9904	0,8957	0,953
Year 1	0,7099	0,7893	0,686	0,5872	0,6047	0,749
Year 2	0,6436	0,8487	0,7218	0,6735	0,5692	0,8003
Year 3	0,7899	0,9485	0,9545	0,7186	0,5949	0,7813
Year 4	0,8102	0,9603	0,985	0,515	0,545	0,545

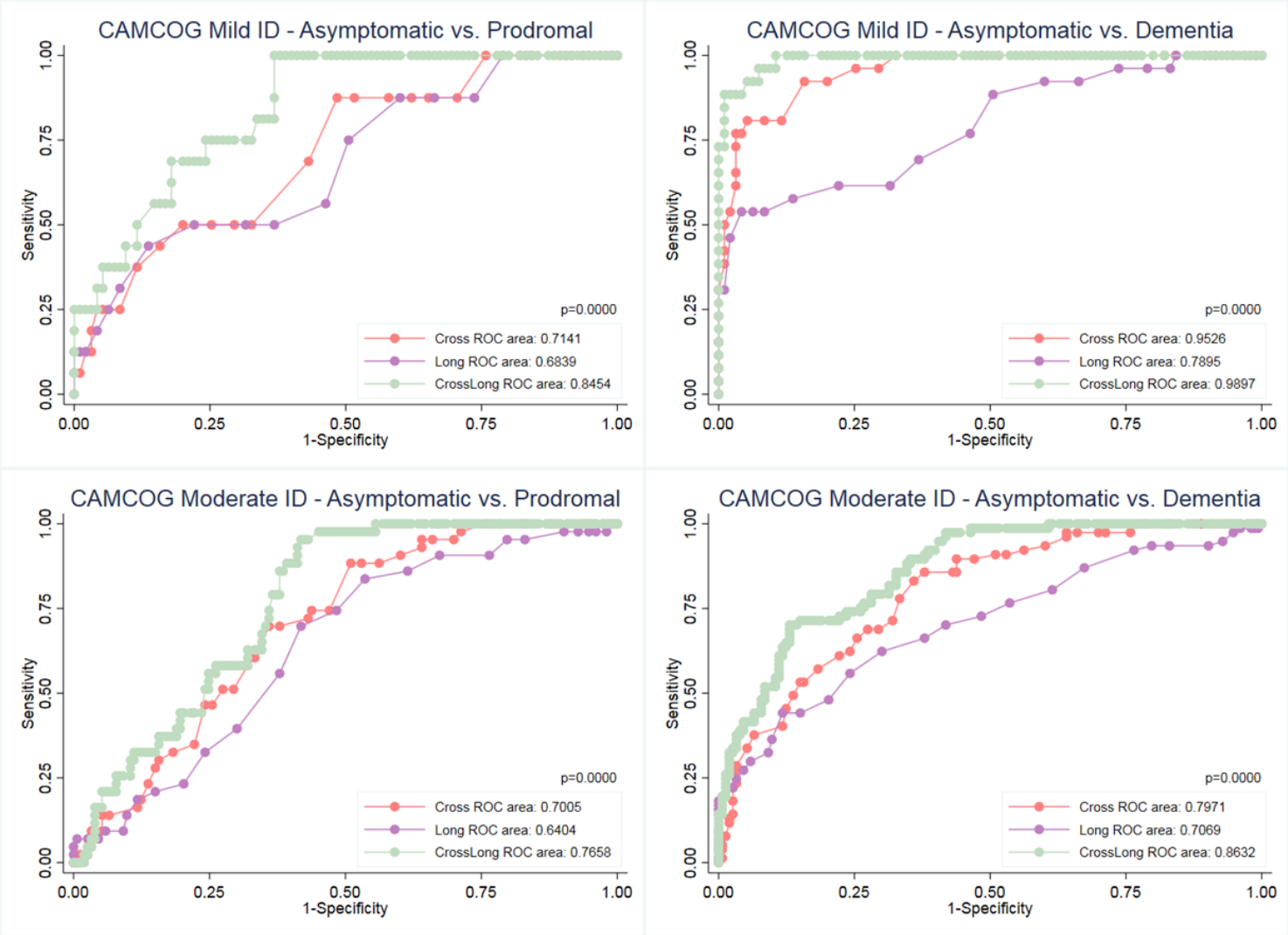
	AUC Raw difference with respect to baseline - Moderate ID					
	CAMCOG-DS		CRT TIR		CRT FIR	
	pDS	dDS	pDS	dDS	pDS	dDS
Baseline	0,7108	0,8537	0,8299	0,937	0,787	0,8912
Year 1	0,6273	0,7025	0,5989	0,6459	0,5794	0,6714
Year 2	0,7137	0,9159	0,7008	0,6246	0,8741	0,7701
Year 3	0,8361	0,9352	0,8776	0,9558	0,7765	0,8376
Year 4	0,5	0,9428	1,000	1,000	0,9565	0,9517

	AUC Raw difference with respect to baseline - All ID					
	CAMCOG-DS		CRT TIR		CRT FIR	
	pDS	dDS	pDS	dDS	pDS	dDS
Baseline	0,7137	0,8532	0,8372	0,9542	0,821	0,9117
Year 1	0,6496	0,7231	0,6175	0,6243	0,5853	0,697
Year 2	0,6998	0,8883	0,7045	0,6557	0,7832	0,7847
Year 3	0,8091	0,9411	0,9009	0,8375	0,7124	0,7966
Year 4	0,8531	0,946	0,9895	0,7958	0,7227	0,7353

Supplementary figure 2: Spaghetti plots for the longitudinal trajectories of cognitive scores by clinical group



Supplementary figure 3: CAMCOG-DS diagnostic performance of the combination of baseline scores and annual change.



Supplementary figure 4: mCRT diagnostic performance of the combination of baseline scores and annual change.