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RESEARCH ARTICLE

Basal forebrain atrophy along the Alzheimer's disease continuum in adults with Down syndrome

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Abstract

Background: Basal forebrain (BF) degeneration occurs in Down syndrome (DS)-associated Alzheimer's disease (AD). However, the dynamics of BF atrophy with age and disease progression, its impact on cognition, and its relationship with AD biomarkers have not been studied in DS.

Methods: We included 234 adults with DS (150 asymptomatic, 38 prodromal AD, and 46 AD dementia) and 147 euploid controls. BF volumes were extracted from T-weighted magnetic resonance images using a stereotactic atlas in SPM12. We assessed BF volume changes with age and along the clinical AD continuum and their relationship to cognitive performance, cerebrospinal fluid (CSF) and plasma amyloid/tau/neurodegeneration biomarkers, and hippocampal volume.

Results: In DS, BF volumes decreased with age and along the clinical AD continuum and significantly correlated with amyloid, tau, and neurofilament light chain changes in CSF and plasma, hippocampal volume, and cognitive performance.

Discussion: BF atrophy is a potentially valuable neuroimaging biomarker of AD-related cholinergic neurodegeneration in DS.

KEYWORDS

Alzheimer's disease, basal forebrain, biomarkers, cholinergic, Down syndrome, magnetic resonance imaging, neuroimaging, volumetry

1 | INTRODUCTION

The basal forebrain (BF) is the center of the cerebral cholinergic system, containing the main cholinergic projection neurons that innervate the limbic structures and the neocortex.[1] Alzheimer's disease (AD) dementia patients exhibit brain deficits in the enzymes involved in acetylcholine synthesis.[2–4] Such reductions correlate with cognitive impairment,[5] and with the loss and atrophy of cholinergic neurons in the BF.[6–8]

Recent magnetic resonance imaging (MRI)-based neuroimaging studies have shown that BF cholinergic degeneration appears early in the clinical AD continuum, with BF volume loss occurring in preclinical and prodromal sporadic AD.[9–13] BF atrophy also correlates with AD biomarkers,[14] and predicts cognitive impairment.[15] Hence, BF atrophy biomarkers could help identify individuals at risk of cognitive deterioration in AD.

Down syndrome (DS) is considered a genetically determined form of AD,[16] and deficits in cortical choline acetyltransferase activity have also been reported in this population.[17] Moreover, neuropathological studies revealed that adults with DS have fewer and smaller cholinergic neurons in the BF compared to age-matched controls.[18,19] In this sense, MRI studies have shown BF and hippocampal atrophy in individuals with DS who progressed from asymptomatic to symptomatic AD.[20]

However, no studies have yet investigated BF morphometric changes along the full clinical AD continuum in DS or their relationship with cognitive performance and AD biomarkers.

In this study, we describe changes in the BF volume with age and along the clinical AD continuum in DS. We further assess continuous associations with cognitive performance, cerebrospinal fluid (CSF) and plasma amyloid/tau/neurodegeneration (AT[N]) biomarkers, and hippocampal atrophy.

2 | METHODS

2.1 | Participants

This is a single-center cross-sectional study of adults with DS and euploid controls from the Down-Alzheimer Barcelona Neuroimaging Initiative (DABNI) and Sant Pau Initiative on Neurodegeneration (SPIN) cohorts in Barcelona, Spain. DABNI is a longitudinal cohort investigating the natural history of AD in DS with multimodal biomarkers.[21] SPIN is a cohort for multimodal biomarker discovery and validation that includes cognitively healthy volunteers and participants with different neurodegenerative diseases.[22]

The study included participants of both sexes (18+ years) with available volumetric T1-weighted MRI sequence (T1w). Exclusion criteria were any significant unstable medical or psychiatric disease affecting cognition and contraindications for MRI (claustrophobia, pacemaker, aneurysm clip, etc.). The Sant Pau Research Ethics Committee approved the study, following the human medical research standards recommended by the Declaration of Helsinki. All participants and/or their legally authorized representatives (in the case of

individuals with DS) gave written informed consent. Participants were recruited between January 2011 and July 2021.

2.2 | Clinical and cognitive evaluation

Intellectual disability (ID) was categorized into mild, moderate, severe, or profound according to the Diagnostic and Statistical Manual of Mental Disorders—Fifth Edition (DSM-V). This assessment was based on caregivers' reports of the individuals' best-ever level of functioning and on the Intelligence Quotient score of the Kaufman Brief Intelligence Test Spanish version when possible.[23] Participants with mild or moderate ID further underwent cognitive assessment with the Cambridge Cognitive Examination adapted for individuals with Down Syndrome and others with intellectual disabilities (CAMCOG-DS, Spanish version)[24,25] and with the modified Cued Recall Test (mCRT),[26] as detailed in previous studies.[21,27–29] The CAMCOG-DS is a cognitive battery that evaluates orientation, language, memory, attention, praxis, abstract thinking, and perception. The mCRT is an adapted test to assess free and cued episodic memory in people with ID.

After independent neurological and neuropsychological evaluations (blinded to biomarker data), each participant with DS was classified in a consensus meeting as asymptomatic (aDS) when there was no clinical or neuropsychological suspicion of AD (i.e., absence of cognitive or functional decline compared to the previous functioning); prodromal AD (pDS) when there was cognitive impairment, but symptoms did not fulfill the criteria for dementia (i.e., cognitive impairment without functional changes); and AD dementia (dDS) when there was a functional decline compared to the previous functioning. The assessment of functional status for differentiating pDS and dDS was based on anamnesis, the Dementia Questionnaire for People with Learning Disabilities, and the Cambridge Examination for Mental Disorders of the Elderly, modified for use assessing people with Down Syndrome(CAMDEX-DS).[28] We also included CSF amyloid-negative euploid controls within the same age range as participants with DS (23 to 65 years), selected from the SPIN cohort.

2.3 | Image acquisition and processing

MRI data were acquired with a 3T Philips Achieva scanner (Philips Healthcare) at Hospital del Mar (Barcelona) or with a 3T Siemens Prisma scanner (Siemens Healthcare) at Hospital Clínic (Barcelona) between 2011 and 2022. Imaging protocols in both scanners included T1w images with 1.0 mm isotropic resolution. The acquisition parameters for each scanner are provided in Table S1 in supporting information.

T1w images were processed with the voxel-based morphometry pipeline implemented in the Computational Anatomy Toolbox (CAT12; Christian Gaser and Robert Dahnke; http://dbm.neuro.uni-jena.de/cat/) for Statistical Parametric Mapping software (SPM12; http://www.fil.ion.ucl.ac.uk/spm/software/spm12/), using the default parameters for voxel-based morphometry.

RESEARCH IN CONTEXT

1. **Systematic Review**: Studies have shown that degeneration of basal forebrain (BF) cholinergic neurons occurs in sporadic Alzheimer's disease (AD) and Down syndrome (DS)-associated AD. However, the dynamic of BF atrophy with age and disease progression, its impact on cognition, and its relationship with AD pathophysiology assessed by fluid biomarkers have not been studied in Down syndrome. Relevant related publications are appropriately cited.

2. **Interpretation**: BF volume decreased with age and along the AD clinical stages and correlated with amyloid/tau/neurodegeneration biomarkers and cognitive performance. Our findings suggest that BF atrophy is a potentially valuable neuroimaging biomarker of AD-related cholinergic neurodegeneration in DS.

3. **Future Directions**: Our findings support the magnetic resonance imaging assessment of BF volume as an early biomarker of AD-related cholinergic neurodegeneration in adults with DS. Hence, as secondary prevention AD trials prepare to include participants with DS, assessment of BF atrophy could help monitor drug efficacy.

The CAT12 voxel-based morphometry pipeline segments T1w images into gray matter (GM), white matter (WM), and CSF maps of 1.5 mm isotropic voxels. Then, each map is registered to standard stereotactic space (Montreal Neurological Institute [MNI]) using Geodesic shooting. This pipeline also automatically extracts hippocampal volumes using the neuromorphometrics atlas, and further estimates total intracranial volumes (TIV).

Additionally, we extracted volumes of the anteromedial (amBF) and posterior (pBF) areas of the BF from the warped and modulated GM map using a recently developed stereotactic atlas of functionally homogeneous BF subdivisions (Figure S1 in supporting information).[30] The amBF corresponds to histopathologically defined cholinergic nuclei 1-3 (Ch1-3), which innervates the hippocampal complex and olfactory bulbs, and the pBF corresponds to cholinergic nucleus 4 (Ch4), which innervates the cerebral (especially limbic and paralimbic) cortex and amygdala.[31,32]

The quality assurance report provided by CAT12 preprocessing (https://neuro-jena.github.io/cat/) was used for assessment of image quality and exclusion of participants with low image quality that could impair adequate volumetric measures of the BF. Participants with an image quality rating <70% were excluded from the study. We found that including T1w images with an image quality rating > 70% would grant acceptable imaging quality and exclude the least number of subjects possible.

To control for head size variability among subjects and between groups, amBF, pBF, and hippocampal volumes were adjusted for TIV

using the residuals method.[33] Briefly, this approach consists of computing a linear regression of the volume of interest and TIV in the control group to obtain the intercept and the slope of the line that fits the data. These parameters are then used to adjust the volume of interest of all participants (controls and with DS). We used the residuals method to minimize the impact of developmental differences in brain size between euploid controls and participants with DS. A simple proportional method to correct BF volumes for TIV overestimates the (relative) BF volumes.

2.4 | CSF and plasma biomarkers acquisition and analyses

A subset of participants underwent lumbar puncture ($n = 147$) with CSF tap and/or blood collection by venipuncture ($n = 212$). Samples were processed as previously described[27] and stored at –80°C before analysis. The CSF levels of amyloid beta 1-40 (Aβ40) and amyloid beta 1-42 (Aβ42) peptides and phosphorylated tau 181 (p-tau181; $n = 138$) were measured using the Lumipulse G600II fully automated platform (Lumipulse, Fujirebio-Europe).[22] CSF neurofilament light chain (NfL) concentration ($n = 147$) was quantified with an enzyme-linked immunosorbent assay (ELISA; NF-Light Assay; UmanDiagnostics), following the manufacturer's recommendations. All CSF samples were analyzed at Hospital Sant Pau, Spain. Plasma concentrations of p-tau181 ($n = 202$) and NfL ($n = 197$) were measured with single molecule array (Simoa) technology (Quanterix). p-tau181 was analyzed at the University of Gothenburg, Sweden, and NfL at Hospital Sant Pau, Spain, following established protocols.[21,27,34] The Alzheimer's laboratory at Hospital Sant Pau integrates the Alzheimer's Association external quality control program for CSF biomarkers.[35]

CSF Aβ42/Aβ40 ratio and p-tau181 levels were used to stratify asymptomatic participants with DS according to the presence of amyloid (A+) and tau (T+) pathologies. Cutoff values of CSF Aβ42/Aβ40 < 0.062 and CSF p-tau181 > 63 pg/mL were used to consider participants as A+ and T+, respectively.[36]

2.5 | Statistical analysis

Statistical analyses were performed with R software, version 3.6.3 (www.R-project.org). Association of amBF and pBF volumes with age were assessed in DS and controls with locally estimated scatterplot smoothing (LOESS) curves, using a first-order LOESS model with a tricubic weight function and a span parameter of 0.75 as previously described.[21] We considered a significant difference when the curves diverged visually rather than when the 95% confidence intervals did not overlap, as the exact age at which the intervals diverge depends on intrinsic limitations of studies assessing the natural history of biomarkers, such as the nature of the variable; the slope of the association curve; and, in our study, the uneven sample sizes.[21]

Data distribution was visually assessed with QQ plots. Differences in amBF, pBF, and hippocampal volumes between controls, aDS, pDS,

and dDS were assessed with Kruskal–Wallis test followed by Dunn's test. The effect size assessment was performed with Cohen's d test. The influence of biological sex and apolipoprotein E (APOE) ε4 carriership on BF atrophy within each study group was assessed with Mann–Whitney test. Correction for multiple comparisons was performed with false discovery rate (FDR).

Spearman correlation analysis was used to assess the association of amBF and pBF volumes with cognitive performance and AT(N) biomarkers in participants with DS. For the correlation with cognitive performance, we used the CAMCOG-DS total scores and the mCRT immediate free recall scores in participants with mild and moderate ID separately. We chose non-parametric tests as we performed several correlation analyses with biomarkers that did not meet the normality assumption. We performed correlation analyses of BF volumes and AT(N) biomarkers and hippocampal volume in aDS, pDS, and dDS participants separately, but also in symptomatic individuals (collapsing pDS and dDS) to increase the statistical power as in previous works.[21]

Data analysis was performed between May and July 2022. Significance was set at $P < .05$.

3 | RESULTS

3.1 | Demographics

Table 1 summarizes the demographic data of study participants. A total of 393 individuals met the inclusion criteria of the study. After quality assessment of T1w images, we included 381 participants: 234 adults with DS (150 aDS, 38 pDS, and 46 dDS) and 147 euploid controls (Figure S2 in supporting information). Controls were older than participants with DS (median age [interquartile range], in years: 53.6 [46.5–58.1] vs. 45.6 [37.2–51.3], $P < .001$), and had a greater proportion of females than the group with DS (73% vs. 42%; $P < .001$). There were no significant differences in the proportion of APOE ε4 carriers between groups.

Among participants with DS, 189 (81%) underwent cognitive assessment, 148 (63%) had CSF biomarkers, and 213 (91%) had plasma biomarker measures. There were no significant differences in the demographics between these subsets and the overall cohort.

3.2 | BF atrophy with age and along the clinical AD continuum

We compared the age-related changes in adjusted amBF, pBF, and hippocampal volumes between adults with DS and euploid controls. amBF and pBF volumetric reduction with age span was more prominent in DS than in controls, starting in the third decade of life (30 years before the median age for prodromal AD diagnosis[21,37]) with a linear decline until ≈age 40 and a steeper decline thereafter (Figures 1A-C). In contrast,

TABLE 1 Study participants.

Sample size N (%)	Controls 147 (39)	aDS 150 (39)	pDS 38 (10)	dDS 46 (12)	P-value NA
Demographics					
Age, median (IQR), years	53.8 (47.3–58)	39.8 (29.8–46.3)	49.2 (47.0–52.5)	53.7 (49.1–56.2)	<.001[a]
Sex (female), N (%)	105 (71)	60 (40)	17 (45)	20 (44)	<.001[b]
APOE ε4 carrier	30 (20)	26 (17)	8 (21)	7 (15)	.728[b]
Mild ID, N (%)	NA	46 (31)	8 (21)	5 (11)	.021[b]
Moderate ID, N (%)	NA	79 (53)	24 (63)	30 (65)	.223[b]
Severe/profound ID, N (%)	NA	25 (17)	6 (16)	11 (25)	<.001[b]
Neuroimaging biomarkers; median (IQR)					
amBF volume (cm³)	1.35 (1.29–1.40)	1.37 (1.30–1.43)	1.23 (1.18–1.29)	1.15 (1.09–1.23)	<.001[a]
pBF volume (cm³)	1.02 (0.98–1.08)	1.12 (1.07–1.20)	1.02 (0.91–1.08)	0.89 (0.82–0.96)	<.001[a]
Hippocampal volume (cm³)	6.3 (6.1–6.5)	5.8 (5.4–6.0)	5.2 (4.8-5.4)	4.1 (3.5–4.7)	<.001[a]
Cognitive test scores; median (IQR)					
CAMCOG-DSTotal score (n = 189)	NA	72 (61–85)	70.5 (58–76.75)	52 (35–58)	<.001[a]
mCRT immediate free recall scores (n = 158)	NA	19 (15–21)	12 (8–14)	7 (4–10)	<.001[a]
CSF biomarkers; median (IQR)					
Aβ₄₂/Aβ₄₀ (n = 225)	0.1 (0.1–0.1)	0.08 (0.06–0.09)	0.04 (0.04–0.05)	0.04 (0.04–0.05)	<.001[a]
p-tau-181 (pg/mL, n = 225)	33 (28–76)	28 (17–44)	96 (54–167)	130 (93–174)	<.001[a]
NfL (pg/mL, n = 268)	365 (300–488)	366 (232–488)	734 (651–1094)	1201 (749–1870)	<.001[a]
Plasma biomarkers; median (IQR)					
p-tau-181 (pg/mL, n = 248)	12 (9–16)	11 (8–16)	20 (12–25)	24 (18–40)	<.001[a]
NfL (pg/mL, n = 314)	8 (6–11)	9 (6–14)	15 (11–22)	26 (19–38)	<.001[a]

Notes: Data of hippocampal volume, amBF volume, and pBF volume presented in the table are adjusted for total intracranial volume.

Abbreviations: $A\beta_{42}/A\beta_{40}$, amyloid beta 1-42/amyloid beta 1-40 ratio; aDS, asymptomatic Down syndrome; amBF, anteromedial basal forebrain; CAMCOG-DS, Cambridge Examination for Mental Disorders of Older People with Down Syndrome and others with intellectual disabilities; CSF, cerebrospinal fluid; dDS, participants with Down syndrome and Alzheimer's disease dementia; ID, intellectual disability; IQR, interquartile range; mCRT, modified cued recall test; NA, not applicable; NfL, neurofilament light chain; pBF, posterior basal forebrain; pDS, participants with Down syndrome and prodromal Alzheimer's disease; p-tau-181, phosphorylated tau-181.

[a]Kruskal-Wallis test.

[b]Chi-squared test.

NA = not applicable.

hippocampal volume (which had a lower offset) only started to atrophy after age 40, suggesting BF volume changes antedate those of the hippocampi (Figures 1A-C).

There was progressive atrophy in amBF, pBF, and hippocampus with disease progression along the AD clinical continuum (Figures 1D-F). pDS had lower BF volumes than aDS ($P_{FDR} < 0.001$, $d = 1.5$) and dDS than with both aDS ($P_{FDR} < 0.001$, $d = 2.1$) and pDS groups ($P_{FDR} = 0.027$, $d = 0.6$). Likewise, lower hippocampal volumes were also observed in pDS compared to aDS ($P_{FDR} < 0.001$, d = 1.3) and in dDS compared to both aDS ($P_{FDR} < 0.001$, d = 2.4) and pDS groups ($P_{FDR} < 0.001$, d = 1.2).

We also stratified the analyses by biological sex and APOE ε4 status. aDS males had higher pBF volumes than females ($P_{FDR} = 0.047$; Figure S3 in supporting information). The APOE ε4 haplotype did not influence amBF or pBF volume loss with age or along the AD continuum in DS (Figure S4 in supporting information).
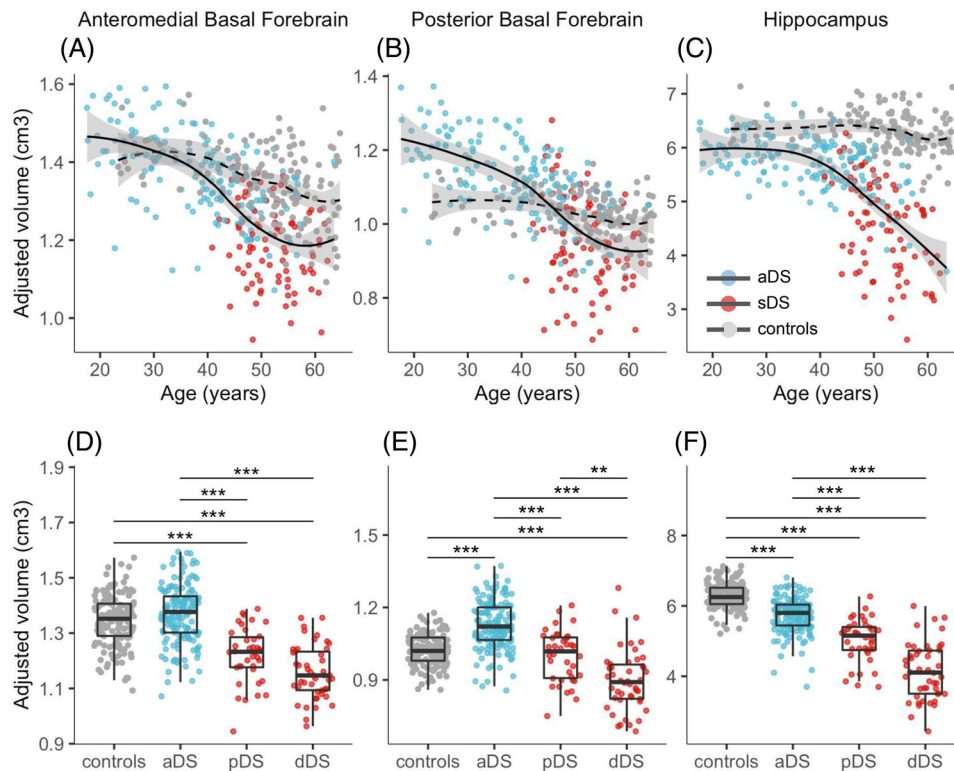
FIGURE 1 Basal forebrain volume across age and along the Alzheimer's disease continuum in Down syndrome. Volumes of basal forebrain and hippocampus are adjusted for the total intracranial volume. Significance values expressed as *$P = 0.05$, **$P = 0.01$, ***$P = 0.001$ (Dunn's test; false discovery rate corrected). Shading represents 95% confidence intervals. aDS, asymptomatic Down syndrome; dDS, Down syndrome-related Alzheimer's disease dementia; pDS, prodromal Down syndrome-related Alzheimer's disease; sDS, symptomatic Down syndrome (pDS + dDS).

3.3 | BF atrophy and cognitive decline in DS

Volumes of amBF and pBF correlated significantly with global cognition and episodic memory, as reflected by the correlations with CAMCOG-DS total scores and with mCRT immediate free recall scores. In participants with mild ID, we found significant associations between amBF and pBF volumes with episodic memory but not with global cognition scores. Among participants with moderate ID, both CAMCOG-DS and mCRT scores correlated significantly with amBF and pBF volumes. Correlations were stronger for episodic memory than global cognition in both groups (Figure 2).

3.4 | Relationship of BF volume with AT(N) biomarkers and hippocampal volume

In adults with DS, BF atrophy showed significant correlations with AT(N) biomarkers (Figure 3). Volumes of amBF and pBF correlated with the CSF Aβ42/40 ratio (lower volumes with lower Aβ42/40 ratio), with p-tau181 and NfL (lower volumes with higher p-tau181 and NfL), and with hippocampal volume (Figure 3 and Table S2 in supporting information). BF volumes also significantly correlated with plasma p-tau181 and NfL (Table S2).

When participants were stratified according to the presence or absence of AD symptoms, there was a significant correlation of amBF and pBF volumes with the CSF Aβ42/40 ratio in asymptomatic but not in symptomatic participants (Table S2). Correlations between amBF and pBF volumes and CSF p-tau181, plasma p-tau181, CSF NfL, and plasma NfL remained significant in both asymptomatic and symptomatic participants (Table S2). Further stratification of aDS individuals according to CSF tau levels into T− and T+ subgroups showed that amBF and pBF volumes correlated with CSF NfL even in T- participants (Figure 4).

4 | DISCUSSION

This study showed that BF atrophy in adults with DS starts very early in the third decade of life, being one of the earliest biomarkers to show changes in adults with DS.[21] There was a steeper decline after age 40 in parallel with the hippocampal atrophy and with disease progression along the AD continuum. Furthermore, BF atrophy was associated with worse global cognition and episodic memory in adults with DS. We also showed for the first time that BF atrophy is associated with amyloid (in asymptomatic individuals only), tau, and neurofilament light changes.
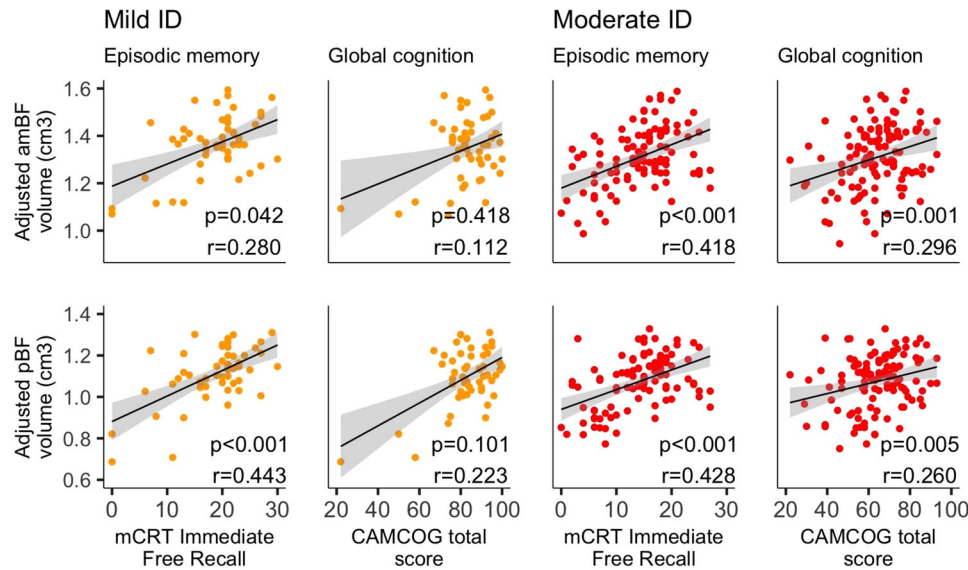
FIGURE 2 Correlations between basal forebrain volumes and memory assessment tests. Volumes amBF and pBF are adjusted for the total intracranial volume (TIV). P and r values obtained with Spearman correlation analysis. amBF, anteromedial basal forebrain; CAMCOG-DS, Cambridge Examination for Mental Disorders of Older People with Down's Syndrome; ID = intellectual disability; mCRT, modified Cued Recall Test; pBF, posterior basal forebrain.

FIGURE 3 Correlations between basal forebrain volumes and AT(N) biomarkers. Volumes of amBF and pBF, and hippocampal volume are adjusted for the total intracranial volume (TIV). P and r values obtained with Spearman correlation. A, amyloid; Aβ, amyloid beta; amBF, anteromedial basal forebrain; CSF, cerebrospinal fluid; N, neurodegeneration; NfL, neurofilament light chain; pBF, posterior basal forebrain; p-tau, phosphorylated tau; T, tau.
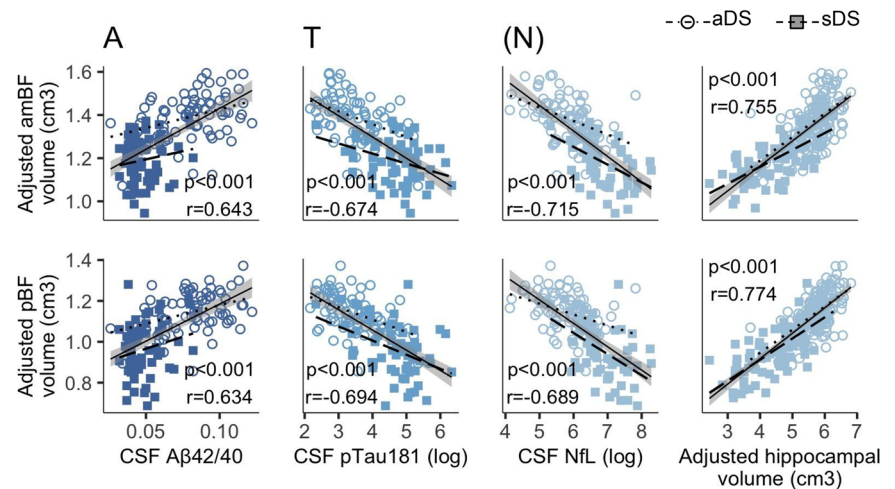
The very early decline in BF volumes in the third decade occurs together with the initial decline in CSF Aβ42/40 and increase in plasma NfL values, which were found (earlier than expected) in the late 20s, before the elevations in CSF p-tau or hippocampal atrophy.[21] Interestingly, in asymptomatic participants with DS, amBF and pBF atrophy were related to CSF NfL levels even before the detection of tau pathology, suggesting amyloid-related neurodegeneration. BF atrophy thus starts >20 years before the mean age of diagnosis of prodromal AD.[21,37] However, we found a clear acceleration beyond age 40 in parallel with hippocampal atrophy and cognitive decline.[21] This acceleration thus occurs when there is both amyloid and tau deposition, suggesting a synergistic effect in cholinergic neurodegeneration.[21,38] Of note, when stratifying participants into asymptomatic versus symp-

tomatic, we found that the association between BF atrophy and CSF Aβ42/40 ratio was significant only among asymptomatic subjects, while the association between BF atrophy and p-tau181 was stronger in symptomatic participants. Interestingly, there was a correlation between NfL levels and BF volumes in T– individuals, supporting BF atrophy's link to neurodegeneration before tau positivity. Most evidence of the association between amyloidosis and cholinergic degeneration comes from animal and neuropathological studies,[39,40] but less from in vivo research. While MRI and amyloid-positron emission tomography (PET) studies in cognitively unimpaired adults, mild cognitive impairment, and sporadic AD dementia have shown that BF atrophy was related to the cortical Aβ burden,[11,14,41] others have found that BF atrophy on MRI was related to abnormal levels of p-tau181, but

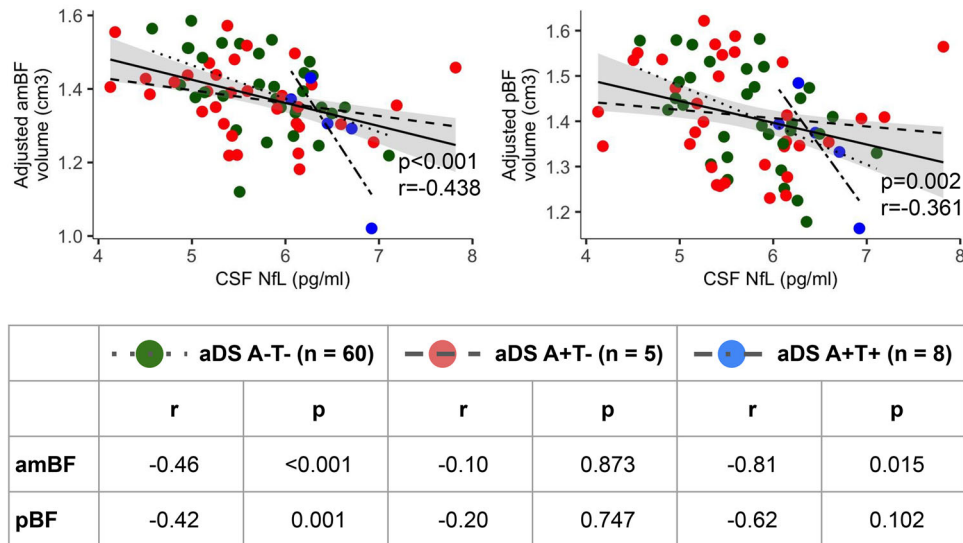|	· · ● · · aDS A-T- (n = 60)		— ● — aDS A+T- (n = 5)		— ● — aDS A+T+ (n = 8)	
	r	p	r	p	r	p
amBF	-0.46	<0.001	-0.10	0.873	-0.81	0.015
pBF	-0.42	0.001	-0.20	0.747	-0.62	0.102

FIGURE 4 Correlations between basal forebrain volumes and CSF NfL in asymptomatic Down syndrome according to tau status. amBF = anteromedial basal forebrain; pBF = posterior basal forebrain; CSF: cerebrospinal fluid; NfL: neurofilament light. Volumes of amBF and pBF are adjusted for the total intracranial volume (TIV). *P* and *r* values obtained with Spearman correlation. A, amyloid; aDS, asymptomatic Down syndrome; amBF, anteromedial basal forebrain; CSF, cerebrospinal fluid; NfL, neurofilament light chain; pBF, posterior basal forebrain; p-tau, phosphorylated tau; T, tau.

not of Aβ1-42 in the CSF.[13] AD pathophysiology affects nerve growth factor (NGF) metabolism, compromising the trophic support to BF neurons even at preclinical AD stages, as shown both in pathological[42] and CSF studies.[43] Of note and as expected, given the strong interconnections between the structures, amBF and pBF also correlated with hippocampal volume.[1,32]

We have also observed higher pBF volumes in men than in women with aDS and no influence of *APOE ε*4 in BF volumes or AD-related changes. The brain volume (and subcortical structures) is higher in men than in women.[44] However, studies reporting sex differences in the BF have been discrepant. Neuropathology studies have shown more AD-related cytoskeletal alterations in the nucleus basalis of Meynert in women than men,[45] while a recent neuroimaging meta-analysis found no sex differences in BF volume of the general population.[46] Regarding APOE genotype, we recently showed an impact of the *APOE ε*4 haplotype in some biomarkers of neurodegeneration ([18F]fluorodeoxyglucose PET and hippocampal volume), but not in others (NfL levels).[47] Finally, BF atrophy was associated with worse global cognition and episodic memory in participants with moderate ID but only with worse episodic memory in participants with mild ID. This result is probably due to ceiling effects in the CAMCOG-DS in participants with mild ID.[28]

Our findings have important implications. They support the MRI assessment of BF volume as an early biomarker of AD-related cholinergic neurodegeneration in adults with DS. Recently, Teipel et al.[15] have shown that BF volume predicted global cognitive decline in individuals with sporadic AD treated with cholinesterase inhibitors. In that study, BF volume showed higher accuracy than hippocampal volume in discriminating cognitive decliners from non-decliners, signaling the need to study the potential of BF volume in predicting response

to cholinergic treatment.[15] Cholinesterase inhibitors are the main pharmacological therapy for cognitive symptoms in AD, improving cognitive and behavioral manifestations[48] and reducing the risk of patient institutionalization[49] and the burden on the caregivers.[50] The results of this paper underscore the cholinergic deficits in adults with DS and support the common clinical practice of using cholinesterase inhibitors (but not memantine) in this population.[16] As secondary prevention AD trials prepare to include participants with DS, assessment of BF atrophy could help monitor drug efficacy.

The main strength of our work is studying a large, well-characterized, and population-based cohort of adults with DS with clinical and multimodal AD biomarkers, allowing us to perform an in-depth analysis of the relationship of BF atrophy with AT(N) biomarkers and cognitive performance in relation to AD clinical stages. Moreover, we have used a mask to extract BF volumes based on a functional parcellation of the BF that defines subregions in a data-driven manner and thus does not depend on subjective manual definitions of these subregions according to some external anatomical landmarks.[30] Our study also has limitations. Because cholinergic nuclei are not directly visible in structural MRI data and indirectly localized through the stereotactic atlas, atrophy in the anterior and posterior BF segments might reflect not only degeneration of cholinergic neurons but also abnormalities in other neuronal populations within the same anatomic region, such as GABAergic and glutamatergic interneurons.[51] Moreover, selecting participants based on the quality of T1w images might introduce bias. Even though only 3% of scans were excluded due to low image quality, this could favor the selection of participants with better cognitive function and, likely, lower AD-related pathological changes. On the other hand, being more permissive with imaging quality problems would have introduced noise to the BF volume

estimates, reducing its accuracy. Finally, this is a cross-sectional study, although the uniform development of AD pathology and its consistent age at onset (between 50 and 55 years) in DS[37] allows the investigation of biomarker temporality pseudo-longitudinally as in autosomal dominant AD.

5 | CONCLUSIONS

In adults with DS, the volume of anteromedial and posterior segments of the BF decreases with age and across clinical stages of AD, and correlates with cognitive performance and AD biomarkers. BF atrophy may be a potentially useful neuroimaging biomarker of AD-related cholinergic neurodegeneration in DS.

AUTHOR CONTRIBUTIONS

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CONFLICTS OF INTEREST AND DISCLOSURE STATEMENT

DA reported receiving personal fees for advisory board services and/or speaker honoraria from Fujirebio-Europe, Roche, Nutricia, Krka Farmacéutica, and Esteve outside the submitted work. AL has served as a consultant or on advisory boards for Fujirebio-Europe, Roche, Biogen, Grifols, and Nutricia, outside the submitted work. JF reported receiving personal fees for service on the advisory boards, adjudication committees, or speaker honoraria from AC Immune, Alzheon, Novartis, Lundbeck, Roche, Fujirebio, NovoNordisk, Esteve, Laboratorios Carnot, Adamed and Biogen, outside the submitted work. DA, AL, and JF report holding a patent for markers of synaptopathy in neurodegenerative disease (licensed to ADx, EPI8382175.0). HZ has served on scientific advisory boards and/or as a consultant for Abbvie, Acumen, Alector, ALZPath, Annexon, Apellis, Artery Therapeutics, AZTherapies, CogRx, Denali, Eisai, Nervgen, Novo Nordisk, Optoceutics, Passage Bio, Pinteon Therapeutics, Red Abbey Labs, reMYND,

Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave; has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure, Biogen, and Roche; and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work). MRA has provided paid consultancy for Veranex. MFI is currently an Altoida Inc employee and holder of stock options (August 2022-present), and has provided paid consultancy for Senopi AG (January-May/2022). No other competing interests were reported. Author disclosures are available in the supporting information.

FUNDING INFORMATION

DATA AVAILABILITY STATEMENT

The authors may share de-identified data that underlie the results reported in this article. Data will be available upon receipt of a request detailing the study hypothesis and statistical analysis plan. All requests should be sent to the corresponding authors. The steering committee of this study will discuss all requests and decide, based on the novelty and scientific rigor of the proposal, whether data sharing is appropriate. All applicants will be asked to sign a data access agreement.

REFERENCES

1. Mesulam MM, Mufson EJ, Levey AI, Wainer BH. Cholinergic innervation of cortex by the basal forebrain: cytochemistry and cortical connections of the septal area, diagonal band nuclei, nucleus basalis (substantia innominata), and hypothalamus in the rhesus monkey. *J Comp Neurol.* 1983;214(2):170-197.
2. Bowen DM, Smith CB, White P, Davison AN. Neurotransmitter-related enzymes and indices of hypoxia in senile dementia and other abiotrophies. *Brain.* 1976;99(3):459-496.
3. Davies P, Maloney AJ. Selective loss of central cholinergic neurons in Alzheimer's disease. *Lancet.* 1976;2(8000):1403.
4. Perry EK, Gibson PH, Blessed G, Perry RH, Tomlinson BE. Neurotransmitter enzyme abnormalities in senile dementia. Choline acetyltransferase and glutamic acid decarboxylase activities in necropsy brain tissue. *J Neurol Sci.* 1977;34(2):247-265.
5. Perry EK, Blessed G, Tomlinson BE, et al. Neurochemical activities in human temporal lobe related to aging and Alzheimer-type changes. *Neurobiol Aging.* 1981;2(4):251-256.
6. Whitehouse PJ, Price DL, Struble RG, Clark AW, Coyle JT, Delon MR. Alzheimer's disease and senile dementia: loss of neurons in the basal forebrain. *Science.* 1982;215(4537):1237-1239.
7. Pearson RC, Sofroniew MV, Cuello AC, et al. Persistence of cholinergic neurons in the basal nucleus in a brain with senile dementia of the Alzheimer's type demonstrated by immunohistochemical staining for choline acetyltransferase. *Brain Res.* 1983;289(1-2):375-379.
8. Mufson EJ, Bothwell M, Kordower JH. Loss of nerve growth factor receptor-containing neurons in Alzheimer's disease: a quantitative analysis across subregions of the basal forebrain. *Exp Neurol.* 1989;105(3):221-232.
9. Grothe M, Heinsen H, Teipel S. Longitudinal measures of cholinergic forebrain atrophy in the transition from healthy aging to Alzheimer's disease. *Neurobiol Aging.* 2013;34(4):1210-1220.
10. Grothe M, Zaborszky L, Atienza M, et al. Reduction of basal forebrain cholinergic system parallels cognitive impairment in patients at high risk of developing Alzheimer's disease. *Cereb Cortex.* 2010;20(7):1685-1695.
11. Grothe MJ, Ewers M, Krause B, Heinsen H, Teipel SJ. Alzheimer's disease neuroimaging initiative. Basal forebrain atrophy and cortical amyloid deposition in nondemented elderly subjects. *Alzheimers Dement.* 2014;10:S344-S353. 5 Suppl.
12. Schmitz TW, Nathan Spreng R. Alzheimer's disease neuroimaging initiative. Basal forebrain degeneration precedes and predicts the cortical spread of Alzheimer's pathology. *Nat Commun.* 2016;7:13249.
13. Cantero JL, Atienza M, Lage C, et al. Atrophy of basal forebrain initiates with tau pathology in individuals at risk for Alzheimer's disease. *Cereb Cortex.* 2020;30(4):2083-2098.
14. Kerbler GM, Fripp J, Rowe CC, et al. Basal forebrain atrophy correlates with amyloid β burden in Alzheimer's disease. *Neuroimage Clin.* 2015;7:105-113.
15. Teipel SJ, Cavedo E, Hampel H, Grothe MJ, Alzheimer's Disease Neuroimaging Initiative. Basal forebrain volume, but not hippocampal volume, is a predictor of global cognitive decline in patients with Alzheimer's disease treated with cholinesterase inhibitors. *Front Neurol.* 2018;9:642.
16. Fortea J, Zaman SH, Hartley S, Rafii MS, Head E, Carmona-Iragui M. Alzheimer's disease associated with Down syndrome: a genetic form of dementia. *Lancet Neurol.* 2021;20(11):930-942.
17. Yates CM, Simpson J, Maloney AF, Gordon A, Reid AH. Alzheimer-like cholinergic deficiency in Down syndrome. *Lancet.* 1980;2(8201):979.
18. Casanova MF, Walker LC, Whitehouse PJ, Price DL. Abnormalities of the nucleus basalis in Down's syndrome. *Ann Neurol.* 1985;18(3):310-313.
19. Mann DM, Yates PO, Marcyniuk B. Some morphometric observations on the cerebral cortex and hippocampus in presenile Alzheimer's disease, senile dementia of Alzheimer type and Down's syndrome in middle age. *J Neurol Sci.* 1985;69(3):139-159.
20. Pujol J, Fenoll R, Ribas-Vidal N, et al. A longitudinal study of brain anatomy changes preceding dementia in Down syndrome. *Neuroimage Clin.* 2018;18:160-166.
21. Fortea J, Vilaplana E, Carmona-Iragui M, et al. Clinical and biomarker changes of Alzheimer's disease in adults with Down syndrome: a cross-sectional study. *Lancet.* 2020;395(10242):1988-1997.
22. Alcolea D, Clarimón J, Carmona-Iragui M, et al. The Sant Pau initiative on neurodegeneration (SPIN) cohort: a data set for biomarker discovery and validation in neurodegenerative disorders. *Alzheimers Dement.* 2019;5:597-609.
23. Kaufman AS, Kaufman NL, KBIT2: Kaufmann Brief Intelligence Test. 3rd Ed. Pearson/PsychCorp; 2004.
24. Ball SL, Holland AJ, Huppert FA, Treppner P, Watson P, Hon J. The modified CAMDEX informant interview is a valid and reliable tool for use in the diagnosis of dementia in adults with Down's syndrome. *J Intellect Disabil Res.* 2004;48:611-620. Pt 6.
25. Esteba-Castillo S, Dalmau-Bueno A, Ribas-Vidal N, Vilà-Alsina M, Novell-Alsina R, García-Alba J. Adaptation and validation of CAMDEX-DS (Cambridge examination for mental disorders of older people with Down's syndrome and others with intellectual disabilities) in Spanish population with intellectual disabilities. *Rev Neurol.* 2013;57(8):337-346.
26. Devenny DA, Zimmerli EJ, Kittler P, Krinsky-McHale SJ. Cued recall in early-stage dementia in adults with Down's syndrome. *J Intellect Disabil Res.* 2002;46:472-483. Pt 6.
27. Fortea J, Carmona-Iragui M, Benejam B, et al. Plasma and CSF biomarkers for the diagnosis of Alzheimer's disease in adults with Down syndrome: a cross-sectional study. *Lancet Neurol.* 2018;17(10):860-869.
28. Benejam B, Videla L, Vilaplana E, et al. Diagnosis of prodromal and Alzheimer's disease dementia in adults with Down syndrome using neuropsychological tests. *Alzheimers Dement.* 2020;12(1):e12047.
29. Videla L, Benejam B, Pegueroles J, et al. Longitudinal clinical and cognitive changes in the Alzheimert's disease continuum in Down syndrome. *JAMA Netw Open.* 2022;5(8):e2225573.
30. Fritz HCJ, Ray N, Dyrba M, Sorg C, Teipel S, Grothe MJ. The corticotopic organization of the human basal forebrain as revealed by regionally selective functional connectivity profiles. *Hum Brain Mapp.* 2019;40(3):868-878.

31. Mesulam MM. Cholinergic circuitry of the human nucleus basalis and its fate in Alzheimer's disease. *J Comp Neurol.* 2013;521(18):4124-4144.

32. Mesulam MM, Mufson EJ, Wainer BH, Levey AI. Central cholinergic pathways in the rat: an overview based on an alternative nomenclature (Ch1-Ch6). *Neuroscience.* 1983;10(4):1185-1201.

33. Voevodskaya O, Simmons A, Nordenskjöld R, et al. The effects of intracranial volume adjustment approaches on multiple regional MRI volumes in healthy aging and Alzheimer's disease. *Front Aging Neurosci.* 2014;6:264.

34. Karikari TK, Pascoal TA, Ashton NJ, et al. Blood phosphorylated tau 181 as a biomarker for Alzheimer's disease: a diagnostic performance and prediction modelling study using data from four prospective cohorts. *Lancet Neurol.* 2020;19(5):422-433.

35. Mattsson N, Andreasson U, Persson S, et al. CSF biomarker variability in the Alzheimer's association quality control program. *Alzheimers Dement.* 2013;9(3):251-261.

36. Alcolea D, Pegueroles J, Muñoz L, et al. Agreement of amyloid PET and CSF biomarkers for Alzheimer's disease on Lumipulse. *Ann Clin Transl Neurol.* 2019;6(9):1815-1824.

37. Iulita MF, Garzón Chavez D, Klitgaard Christensen M, et al. Association of Alzheimer disease with life expectancy in people with down syndrome. *JAMA Netw Open.* 2022;5(5):e2212910.

38. Fortea J, Vilaplana E, Alcolea D, et al. Cerebrospinal fluid β-amyloid and phospho-tau biomarker interactions affecting brain structure in preclinical Alzheimer disease. *Ann Neurol.* 2014;76(2):223-230.

39. Wong TP, Debeir T, Duff K, Cuello AC. Reorganization of cholinergic terminals in the cerebral cortex and hippocampus in transgenic mice carrying mutated presenilin-1 and amyloid precursor protein transgenes. *J Neurosci.* 1999;19(7):2706-2716.

40. Boncristiano S, Calhoun ME, Kelly PH, et al. Cholinergic changes in the APP23 transgenic mouse model of cerebral amyloidosis. *J Neurosci.* 2002;22(8):3234-3243.

41. Teipel SJ, Fritz HC, Grothe MJ. Alzheimer's disease neuroimaging initiative. Neuropathologic features associated with basal forebrain atrophy in Alzheimer disease. *Neurology.* 2020;95(10):e1301-e1311.

42. Pentz R, Iulita MF, Ducatenzeiler A, Bennett DA, Cuello AC. The human brain NGF metabolic pathway is impaired in the pre-clinical and clinical continuum of Alzheimers disease. *Mol Psychiatry.* 2021;26(10):6023-6037.

43. Pentz R, Iulita MF, Ducatenzeiler A, et al. Nerve growth factor (NGF) pathway biomarkers in Down syndrome prior to and after the onset of clinical Alzheimer's disease: a paired CSF and plasma study. *Alzheimers Dement.* 2021;17(4):605-617.

44. Li W, van Tol MJ, Li M, et al. Regional specificity of sex effects on subcortical volumes across the lifespan in healthy aging. *Hum Brain Mapp.* 2014;35(1):238-247.

45. Salehi A, Gonzalez Martinez V, Swaab DF. A sex difference and no effect of ApoE type on the amount of cytoskeletal alterations in the nucleus basalis of Meynert in Alzheimer's disease. *Neurobiol Aging.* 1998;19(6):505-510.

46. Liu S, Seidlitz J, Blumenthal JD, Clasen LS, Raznahan A. Integrative structural, functional, and transcriptomic analyses of sex-biased brain organization in humans. *Proc Natl Acad Sci U S A.* 2020;117(31):18788-18798.

47. Bejanin A, Iulita MF, Vilaplana E, et al. Association of apolipoprotein E ε4 allele with clinical and multimodal biomarker changes of Alzheimer disease in adults with down syndrome. *JAMA Neurol.* 2021;78(8):937-947. Published online July 6.

48. Massoud F, Gauthier S. Update on the pharmacological treatment of Alzheimer's disease. *Curr Neuropharmacol.* 2010;8(1):69-80.

49. Feldman HH, Pirttila T, Dartigues JF, et al. Treatment with galantamine and time to nursing home placement in Alzheimer's disease patients with and without cerebrovascular disease. *Int J Geriatr Psychiatry.* 2009;24(5):479-488.

50. Feldman H, Gauthier S, Hecker J, et al. Efficacy of donepezil on maintenance of activities of daily living in patients with moderate to severe Alzheimer's disease and the effect on caregiver burden. *J Am Geriatr Soc.* 2003;51(6):737-744.

51. Anaclet C, Pedersen NP, Ferrari LL, et al. Basal forebrain control of wakefulness and cortical rhythms. *Nat Commun.* 2015;6:8744.

SUPPORTING INFORMATION

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