

Associations of Microbleeds and Their Topography With Imaging and CSF Biomarkers of Alzheimer Pathology in Individuals With Down Syndrome

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Neurology® 2024;103:e209676. doi:10.1212/WNL.0000000000209676

Abstract

Background and Objectives

Cerebral hemorrhages are an exclusion criterion and potential adverse effect of anti-amyloid agents. It is, therefore, critical to characterize the natural history of cerebral microbleeds in populations genetically predisposed to Alzheimer disease (AD), such as Down syndrome (DS). We aimed to assess microbleed emergence in adults with DS across the AD spectrum, defining their topography and associations with clinical variables, cognitive outcomes, and fluid and neuroimaging biomarkers.

Methods

This cross-sectional study included participants aged 18 years or older from the Down-Alzheimer Barcelona Neuroimaging Initiative and Sant Pau Initiative on Neurodegeneration with T1-weighted and susceptibility-weighted images. Participants underwent comprehensive assessments, including apolipoprotein E (APOE) genotyping; fluid and plasma determinations of beta-amyloid, tau, and neurofilament light; cognitive outcomes (Cambridge Cognitive Examination and modified Cued Recall Test); and vascular risk factors (hypertension, diabetes mellitus, and dyslipidemia). We manually segmented microbleeds and characterized their topography. Associations between microbleed severity and AD biomarkers were explored using between-group comparisons (none vs 1 vs 2+) and multivariate linear models.

Results

We included 276 individuals with DS and 158 healthy euploid controls (mean age = 47.8 years, 50.92% female). Individuals with DS were more likely to have microbleeds than controls (20% vs 8.9%, $p < 0.001$), with more severe presentation (12% with 2+ vs 1.9%). Microbleeds increased with age (12% 20-30 years vs 60% > 60 years) and AD clinical stage (12.42% asymptomatic, 27.9% prodromal, 35.09% dementia) were more common in APOEε4 carriers (26% vs 18.3% noncarriers, $p = 0.008$), but not associated with vascular risk factors ($p > 0.05$). Microbleeds were predominantly posterior (cerebellum 33.66%; occipital 14.85%; temporal 21.29%) in participants with DS. Associations with microbleed severity were found for neuroimaging and fluid AD

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The Article Processing Charge was funded by the authors.

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e209676(1)

Glossary

AD = Alzheimer disease; ANT = Advanced Normalization Tool; CAA = cerebral amyloid angiopathy; CAMCOG-DS = Cognitive Examination for Older Adults with Down Syndrome; DS = Down syndrome; ID = intellectual disability; mCRT = modified Cued Recall Test; ROI = regions of interest; SPIN = Sant Pau Initiative on Neurodegeneration; TIV = total intracranial volume; WMH = white matter hyperintensity.

biomarkers, but only hippocampal volumes (standardized $\beta = -0.18 [-0.31, -0.06]$, $p < 0.005$) and CSF p-tau-181 concentrations ($\beta = 0.26 [0.12, 0.41]$, $p < 0.005$) survived regression controlling for age and disease stage, respectively. Microbleeds had limited effect on cognitive outcomes.

Discussion

In participants with DS, microbleeds present with a posterior, lobar predominance, are associated with disease severity, but do not affect cognitive performance. These results suggest an interplay between AD pathology and vascular lesions, implicating microbleeds as a risk factor limiting the use of anti-amyloid agents in this population.

Introduction

In Down syndrome (DS), the triplication of the amyloid precursor protein (*APP*; OMIM 104760) gene is both necessary and sufficient for the emergence of Alzheimer disease (AD) pathology.¹ Beta amyloid ($A\beta$) accumulation starts in adolescence, and virtually all individuals with DS have the neuropathologic hallmarks of AD at age 40.² $A\beta$ can also aggregate within the walls of leptomeningeal and cortical vessels, causing cerebral amyloid angiopathy (CAA).³ This phenomenon, more frequent in individuals with DS than in sporadic AD or healthy ageing,^{4,5} weakens the vessel walls and results in ruptures that leak blood into the parenchyma.³ This manifests on MRI as small hypointense foci on susceptibility-weighted images (SWIs) called microbleeds.⁶

Microbleeds have different topography according to their aetiology. Hypertension preferentially affects arteries in deep areas (basal ganglia, thalamus, and brainstem) and causes deep microbleeds. CAA preferentially affects small arteries and arterioles in lobar areas (cerebral cortex, gray-white matter junctions) which leads most often to lobar microbleeds.⁷ Anti-amyloid agents can also cause microhemorrhages, known as amyloid-related imaging abnormalities with hemosiderosis/microhemorrhages (ARIA-H).⁸ These lesions closely resemble the typical microbleeds occurring outside the context of amyloid removal and can lead to increased monitoring requirements, suspension, or even discontinuation of treatment. With the arrival of anti-amyloid monoclonal antibodies in clinical practice, it is critical to meticulously characterize the natural history of cerebral microbleeds in populations that will directly benefit from the treatment, particularly those individuals genetically predisposed to develop AD.

To date, only a few studies have assessed microbleeds in adults with DS and detected these lesions as early as the fifth decade of life,⁹ and at higher frequency than in healthy controls⁹ or sporadic AD.⁴ However, little is known about the topography of microbleeds in individuals with DS and their

associations with CSF biomarkers and cognitive measures. Using one of the largest cohorts with multimodal AD biomarkers, this study aims to provide a comprehensive assessment of microbleeds, their relationship with AD biomarkers and cognitive outcomes, and their topography.

Methods

Cohort Study Design and Participants

This cross-sectional study included adults with DS from the single-center Down-Alzheimer Barcelona Neuroimaging Initiative (DABNI) and cognitively unimpaired euploid controls from the Sant Pau Initiative on Neurodegeneration (SPIN). DABNI participants are aged 18 years or older with trisomy 21, and SPIN includes control participants without memory complaints or impairment on global cognitive tests, confirmed by comprehensive neuropsychological examination.¹⁰ In both, participants must be able to complete neuropsychological tests and are proposed to undergo MRI, blood draw, and lumbar puncture. The presence of major neurologic or psychiatric diseases is an exclusion criterion.¹⁰ In this study, we included only participants with brain 3T T1-weighted and susceptibility-weighted (SWI) magnetic resonance imaging (MRI). A subset of individuals completed genetic screening for *APOE* genotype ($n = 262$) and collection of vascular risk factor (VRF) data (hypertension, dyslipidemia $n = 272$, and diabetes mellitus $n = 271$).^{10,11}

Diagnostic classification was performed independently by the neuropsychologists and neurologists/psychiatrists assessing participants and discussed during consensus meetings masked to biomarkers. Functional status, differentiating prodromal, and AD dementia were assessed using anamnesis, Dementia Questionnaire for Persons with Mental Retardation, and the CAMDEX-DS.¹² This distinguished cognitive decline from preexisting intellectual disability (ID), with emphasis on establishing change from the individual's best level of functioning. A diagnosis of 'asymptomatic' (aDS) was given when there was no clinical or

neuropsychological suspicion of clinical AD (absence of cognitive impairment beyond ID or functional decline compared with previous functioning); 'prodromal AD' (pDS) for suspicion of AD, but with symptoms not fulfilling criteria for dementia (cognitive impairment without functional changes); or 'AD dementia' (dDS) for evidence of cognitive impairment beyond ID, interfering with everyday activities (i.e., functional decline compared with previous functioning). *The Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition*, was used to stratify the level of ID (mild, moderate, severe/profound) based on individuals' best-ever level of functioning.¹³

Individuals with DS underwent cognitive testing, which included the Cognitive Examination for Older Adults with Down Syndrome (CAMCOG-DS),¹² which assesses orientation, language, memory, attention, praxis, abstract thinking, and perception in the context of aging. In addition, the modified Cued Recall Test (mCRT)¹⁴ was used to evaluate episodic memory. Both tests are adapted for people with ID and validated in the Spanish population. In this study, we used the total CAMCOG-DS score and both the immediate and delayed recall of the mCRT (sum of the free and cued performances for each). To account for the effect of ID on cognitive performances, we excluded severe/profound cases to prevent floor effects and used a z-transformation on each test in mild and moderate ID groups separately.

Image Acquisition Protocol

All participants underwent an MRI imaging protocol at Hospital del Mar (3T Philips-Achieva scanner) or Hospital Clinic (3T Siemens-Prisma), including high-resolution three-dimensional T1-weighted, SWI, and fluid attenuated inversion recovery (FLAIR) sequences. Acquisition protocols are detailed in eTable 1.

Image Processing and Segmentation

Using the Computational Anatomy Toolbox (CAT12),¹⁵ T1-weighted MRIs were preprocessed and total gray matter volume, total intracranial volume (TIV), and bilateral hippocampal volumes (using the Hammers atlas)¹⁶ were extracted. FLAIR images were coregistered to their corresponding T1-weighted image using Advanced Normalization Tools (ANTs; v. 2.3.4),¹⁷ and white matter hyperintensity (WMH) volumes were segmented using the Lesion Prediction Algorithm (LPA)¹⁸ implemented in the Lesion Segmentation Toolbox¹⁹ for SPM12. SWI scans were N4 bias field corrected using ANTs and coregistered to their corresponding T1-weighted image. Microbleed segmentation on the SWIs involved a rigorous protocol with ITK-SNAP (v. 3.8.0)²⁰ developed in consultation with a neuroradiologist (M.A.), wherein raters traced the lesions to create 3D maps for each brain scan. Two independent raters (S.Z. and I.A.) were trained to identify and segment the hypointense spots while blinded to clinical data and other MRI sequences. Visual inspection of the corrected and coregistered SWI scans was completed at the same time. Two images were discarded because of movement artifacts. Two participants, including

one previously described,²¹ were removed because of extensive confluent hemorrhages, that is, when bleeds were not round and isolated, therefore suggesting the possibility of macrobleeds or other lesion source. Raters achieved an interrater reliability of 92.9%, with a quadratic weighted kappa coefficient of 0.796, or substantial agreement according to benchmarks by Landis and Koch.²² Disagreements were resolved with assistance of the neuroradiologist who visually inspected the scan and used other MRI sequences if required to discard microbleed mimics. Results showed the SWI sequences allowed detection of microbleeds of just under 2 mm and above (mean size = 3.8 ± 1.06 mm, range = 1.9–8.4 mm). Overall microbleed number per subject was recorded, and the segmentation resulted in a binary lesion map, allowing quantification of regional microbleeds. Specifically, we determined the number of microbleeds in frontal, temporal, occipital, parietal, and cerebellum regions of interest (ROIs), defined using the Neuromorphometrics atlas (Neuromorphometrics, Inc), implemented in CAT12. To compare results with previous work, we also determined the number of microbleeds in 4 patterns: strictly lobar, strictly deep, mixed (deep and lobar), and cerebellum (with or without lobar microbleeds). Detailed information about all ROIs is provided in eTable 2.

Participants were classified according to the presence of microbleeds [microbleed negative (microbleed–) and microbleed positive (microbleed+)] or severity (0 microbleed, 1 microbleed, and 2+ microbleeds), which is the same as severity ratings used in previous research.^{23,24} Although some research has used different severity classifications, this is the most frequent and is based on evidence for a different phenotype for individuals with multiple microbleeds, typically associated with CAA.^{6,25}

Finally, to visualize the topographic pattern of microbleed, segmented maps were normalized into Montreal Neurologic Institute (MNI) space using normalization parameters of the T1-weighted image. Microbleed normalized maps were then summed together separately for euploid controls and patients with DS, as well as by severity and AD status in the DS population.

Fluid Biomarkers

A subset of individuals with DS and control participants underwent CSF acquisition by lumbar puncture as previously described.^{10,11} Concentrations of CSF amyloid β peptide ($A\beta_{1-40}$) and 1–42 ($A\beta_{1-42}$), total tau (t-tau) and tau phosphorylated at threonine 181 (p-tau-181), were quantified using a commercially available immunoassay on a fully automated platform (Lumipulse; Fujirebio, Europe) following a previously published protocol.¹⁰ CSF neurofilament light chain (NfL) levels were measured with ELISA (NfL Assay; UmanDiagnostics) according to the manufacturer recommendations.

Statistical Analysis

All statistical analyses were conducted with the R software, version 4.0.4 (R Foundation for Statistical Computing).

Differences in baseline characteristics were analyzed using χ^2 tests (or Fisher exact tests, when appropriate) for categorical data. When comparing age, decade age groups were created for the DS group (20s, 30s, etc), while euploid controls were treated as one age group. For continuous variables with normal distribution, 2-sample t-tests (unpaired, 2-tailed) or ANOVA was applied. Mann-Whitney tests or Kruskal-Wallis tests were used when normality assumptions were violated. χ^2 tests were used to assess the effect of microbleed severity on demographic, clinical, and genetic biomarkers. Additional Kruskal-Wallis tests examined the influence of microbleed severity on neuroimaging biomarkers, CSF, and cognitive outcomes. Total gray matter and bilateral hippocampal volumes, divided by TIV, were included as neuroimaging biomarkers of AD neurodegeneration. To control the influence of demographic data, we repeated analyses comparing a group of microbleed+ individuals with DS with a 1:1 group of microbleed- individuals with DS matched for age, sex, and ID using the e1071 R package. We additionally used a sensitivity analysis to assess the effect of microbleed status (microbleed-/microbleed+) on AD neuroimaging biomarkers, CSF and cognitive performances using linear regression analyses. We first performed univariate models, and then regressed out age (model 2) and Alzheimer diagnosis (model 3). The threshold for significance was set at 0.05, and Bonferroni corrections were applied for multiple tests.

Standard Protocol Approvals, Registrations, and Patient Consents

Both DABNI and SPIN were approved by the Sant Pau Ethics Committee following the standards for medical research in humans recommended by the Declaration of Helsinki, and all participants, or their legally authorized representatives, gave written informed consent before enrolment.

Data Availability

The authors may share deidentified, individual participant-level data underlying the reported results. Data will be available on request detailing study hypotheses and statistical analysis plan. The authors will discuss all requests and decide based on the novelty and scientific rigor of the proposal whether data sharing is appropriate. All requests should be sent to the corresponding author. Applicants will be asked to sign a data access agreement.

Results

Characteristics of Study Participants

This study included 276 adults with DS and 158 controls (for details on demographic data, see Table 1). Individuals with DS spanned the whole AD continuum; 161 were aDS, 43 pDS, and 57 dDS. The control group (median age [interquartile range], 56.6 [51.2–63.1]) was significantly older than the DS group (45.7 [37.3–50.9], $p < 0.001$) and included a greater proportion of women (67.1% vs 41.7%, $p < 0.001$). No between-group difference was found in the proportion of *APOE*ε4 allele carriers (27.2% controls, 19% DS, $p = 0.065$) or

diabetes mellitus (4.4% controls and DS), but a higher proportion of controls presented with hypertension (16.5% controls, 2.6% DS) and dyslipidemia (25.9% controls, 17.3% DS, $p < 0.001$).

Associations With Demographic and Clinical Factors

The number and severity of microbleeds differed significantly between participants with DS and controls (20% individuals with DS, 8.9% controls, $p < 0.001$). In individuals with DS, microbleed numbers significantly increased with age (Figure 1A), with 12% having 1 or more microbleeds in their 20s vs 60% of individuals older than 60. The proportion of controls with microbleeds (8.9%) was not significantly different from that of individuals with aDS (12.42%; $p = 0.5$), but the proportion was progressively increased with advancing AD clinical stage, from aDS to pDS (27.9%; $p < 0.05$), and nonsignificantly from pDS to dDS (35.09%; $p = 0.35$; Figure 1B). Following exclusion criteria used in recent AD clinical trials, 6% of the cohort of individuals with DS, including 7% at the prodromal and 12% at the dementia stages, had ≥ 4 microbleeds and would be excluded. These participants had higher amyloid, tau, and neurodegeneration measures, with worse cognitive scores (eTable 3), thus would otherwise fulfill criteria for inclusion in AD clinical trials. In individuals with DS, number and severity of microbleeds did not differ according to sex (Figure 1B) or ID (Figure 1C), but was higher for *APOE*ε4 carriers, because 26% had microbleeds, and of these, 92.3% had 2+ microbleeds compared with noncarriers, 18.3% of which had microbleeds, 51.3% with 2+ microbleeds ($p = 0.007$; Figure 1E). Higher frequency or severity was not observed for individuals with hypertension (microbleeds in 14.3%; 0% with 2+ microbleeds) compared with those without hypertension (20.8%; 12.1% 2+). This was similar in individuals with diabetes mellitus (25%; 8.3% 2+) compared with those without (19.7%; 12% 2+), and with dyslipidemia (25.5%; 15% 2+) vs no dyslipidemia (18.7%; 12% 2+; all $p > 0.05$). Analyses using microbleed presence (microbleed-/microbleed+) rather than microbleed severity demonstrated the same associations (eFigure 1).

Location of Microbleeds

To characterize the topography of microbleeds, we analyzed the location of microbleeds in all participants. Microbleeds were dispersed in controls (Figure 2A) but had an anteroposterior gradient in adults with DS. Microbleeds predominated in the cerebellum (33.66%), occipital (14.85%), and temporal (21.29%) regions. This pattern was apparent and consistent along the AD continuum (Figure 2C). We should note that while 23 adults with DS presented with cerebellar microbleeds, 2 participants accounted for more than 50% of these (38 of 68). These participants did not have the presence of VRFs and both were *APOE*ε3 homozygotes, and when removed, patterns remained highly similar (eFigure 2).

When separated by pattern, strictly lobar microbleeds were common in both controls (68.42%, $n = 13$) and DS (37.25%,

Table 1 Baseline Demographic Characteristics

Characteristics	Controls N = 158	Down syndrome N = 276	p Value	N
Age	56.6 [51.2; 63.1]	45.7 [37.3; 50.9]	<0.001	
Female (%)	106 (67.1)	115 (41.7)	<0.001	
Intellectual disability, n (%)				
Mild	—	78 (28.7)	—	
Moderate	—	150 (55.1)	—	
Severe/profound	—	44 (16.2)	—	
APOEε4 carriers, n (%)	43 (27.2)	50 (19.0)	0.065	
Microbleeds, n (%)			0.005	
0	144 (91.9)	221 (80.1)	—	
1	11 (7.0)	22 (8.0)	—	
2+	3 (1.9)	33 (12.0)	—	
Alzheimer disease clinical stage, n (%)				
Asymptomatic AD	—	161 (61.7)	—	
Prodromal AD	—	43 (16.5)	—	
AD dementia	—	57 (21.8)	—	
Vascular risk factors, n (%)				
Hypertension	26 (16.5)	7 (2.6)	<0.001	387
Diabetes mellitus	7 (4.4)	12 (4.4)	<0.001	386
Dyslipidemia	41 (25.9)	47 (17.3)	<0.001	387
CAMCOG total score	—	68.0 [52.0; 82.0]	—	233
mCRT immediate recall total score	—	34.0 [29.0; 36.0]	—	188
mCRT delayed recall total score	—	17.0 [11.0; 20.0]	—	195
CSF Aβ ₁₋₄₂ /Aβ ₁₋₄₀ ratio	0.10 [0.10; 0.11]	0.06 [0.04; 0.08]	<0.001	284
CSF PTau, pg/mL	35.1 [26.3; 43.7]	48.9 [23.5; 120.3]	0.002	284
CSF Tau, pg/mL	256.0 [192.0; 306.5]	373.0 [213.5; 697.5]	<0.001	285
CSF NfL, pg/mL	369.9 [307.2; 534.7]	523.0 [305.2; 814.5]	<0.001	307

Abbreviations: AD = Alzheimer disease; APOEε4 = apolipoprotein epsilon 4; CAMCOG = Cambridge Cognitive Examination (modified for Down syndrome); mCRT = modified cued recall test; NfL = neurofilament light chain; PTau = phosphorylated tau 181. Values are n (%) or median [IQR].

n = 76), while 59.80% of microbleeds were cerebellar (with or without the presence of lobar MBs) in individuals with DS. In both individuals with DS and controls, strictly deep (5.26%; 0.49%, respectively) and mixed deep and lobar (5.26; 2.45%) microbleeds were rare (eTable 4).

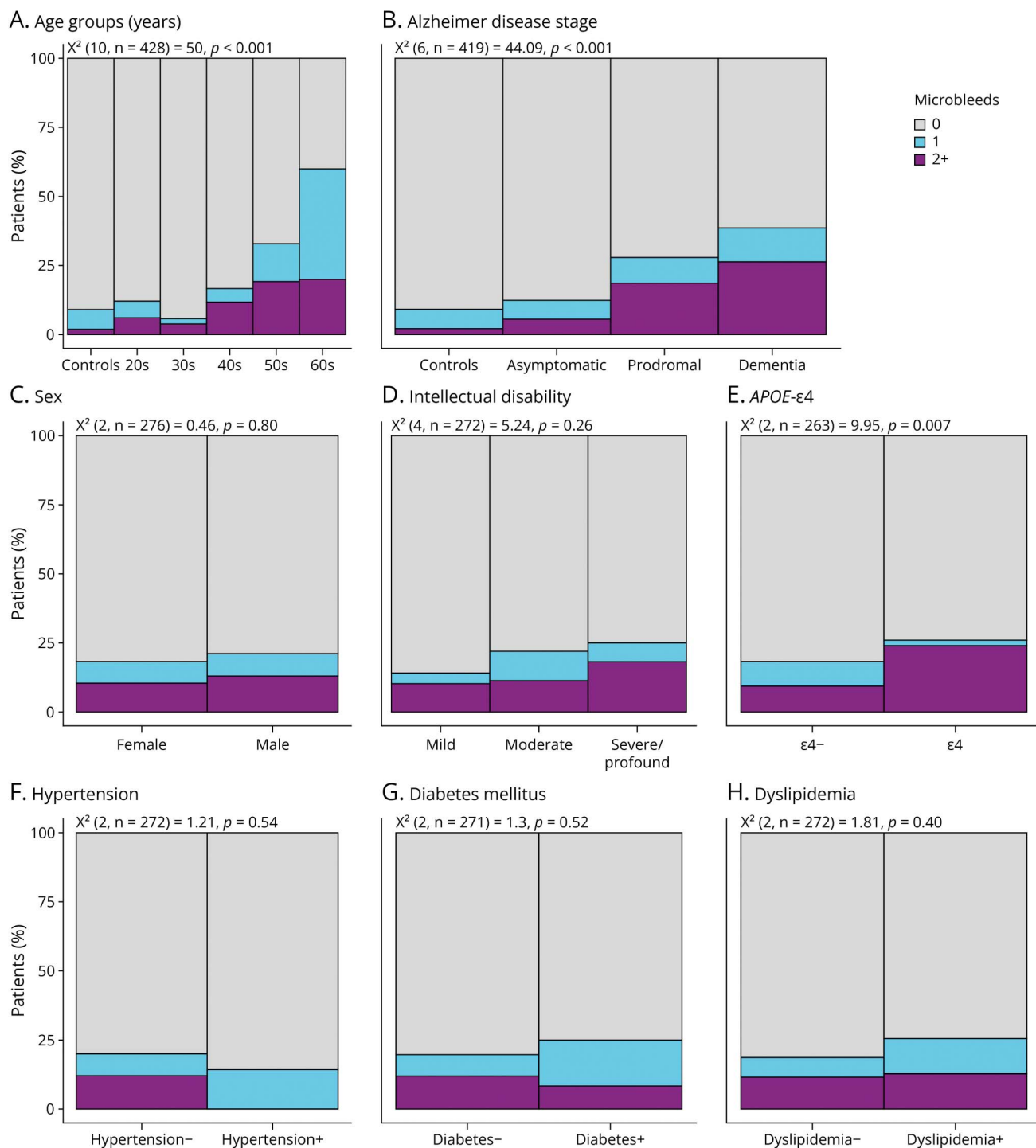
Associations With Imaging and Fluid Biomarkers and Cognitive Outcomes

In participants with DS, increased microbleed severity was associated with increased WMH volume (χ^2 [2, N = 274] = 14.87), decreased hippocampal (χ^2 [2, N = 276] = 18.11) and total gray matter volumes (χ^2 [2, N = 274] = 15.01), lower CSF Aβ₁₋₄₂/Aβ₁₋₄₀ ratio (χ^2 [2, N = 178] = 18.88), and higher concentrations

of CSF p-tau-181 (χ^2 [2, N = 178] = 15.68) and NfL (χ^2 [2, N = 274] = 15.75) (all p < 0.001; Figure 3, A–F). The effect of microbleeds on these measures evolved gradually, but significant differences were mainly observed between the 0 and 2+ microbleed subgroups. Although not surviving Bonferroni correction, individuals with higher microbleed severity tended to demonstrate worse performance on the immediate mCRT scores (χ^2 [2, N = 188] = 7.67, p = 0.02) but not for the CAMCOG (χ^2 [2, N = 233] = 3.35, p = 0.19) or delayed mCRT scores (χ^2 [2, N = 195] = 3.57, p = 0.17; Figure 3, G–I).

Given that microbleeds increased with age and AD clinical stage in participants with DS, we conducted 2 additional

Figure 1 Associations Between Microbleed Severity and Demographic, Genetic, and Clinical Factors

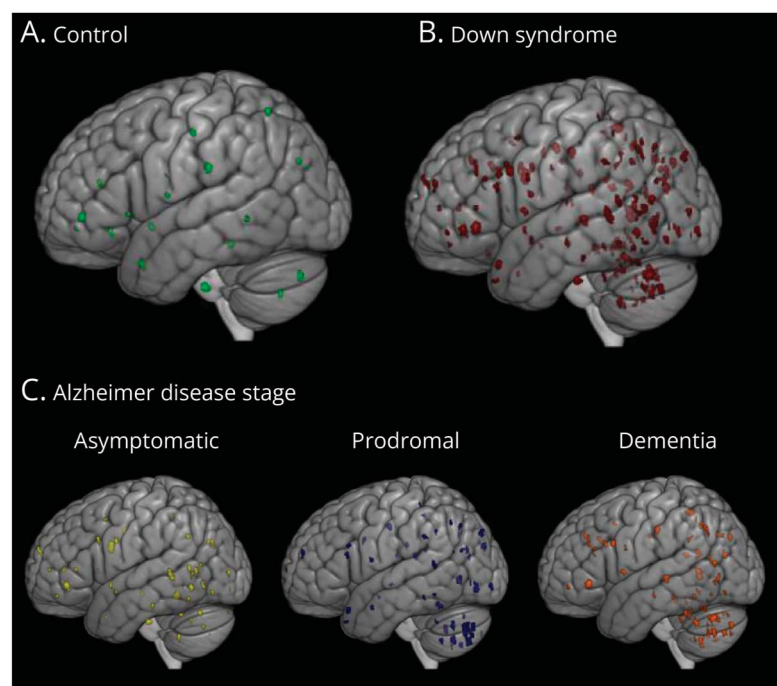


Bar charts representing the proportion of patients by microbleed severity (0, 1, 2+) across demographic, genetic, and clinical factors: In Down syndrome and controls, (A) age in Down syndrome vs controls and (B) Alzheimer disease stage. In Down syndrome only, (C) sex, (D) intellectual disability, (E) *APOE*-ε4, (F) hypertension, (G) diabetes mellitus, and (H) dyslipidemia. *APOE*ε4 = apolipoprotein E epsilon 4; ε4-/+ = *APOE*ε4 noncarrier/carrier.

analyses to establish the specific effect of microbleeds on AD biomarkers and cognition. First, we repeated the previous between-group approach and compared DS microbleed+ with a 1:1 DS microbleed- group matched for age, sex, and ID. There was a loss of statistical significance of group differences for all measures (all $p > 0.05$; eFigure 3). Second, we

performed regression analyses controlling for age and AD diagnosis in the whole cohort of individuals with DS (Figure 4). After Bonferroni correction, regressing out the effects of age (model 2), the presence of microbleeds only remained significantly associated with reduced hippocampal volume (standardized $\beta = -0.18$, 95% CI $[-0.31 \text{ to } -0.06]$, $p <$

Figure 2 Topography of Microbleeds



Rendered MNI space images in MRICron with overlaid summed maps of microbleeds in (A) all controls (green) and (B) Down syndrome (red); (C) Down syndrome by Alzheimer disease clinical stage (asymptomatic = yellow, prodromal = blue, dementia = orange). Transparency denotes depth of microbleed, where lighter microbleeds are deeper in the brain parenchyma.

0.005) and CSF p-tau-181 ($\beta = 0.29$, [0.14–0.44], $p < 0.005$). When regressing out Alzheimer diagnosis (model 2), only the association with CSF p-tau-181 was retained ($\beta = 0.26$, [0.12–0.41], $p < 0.005$).

Discussion

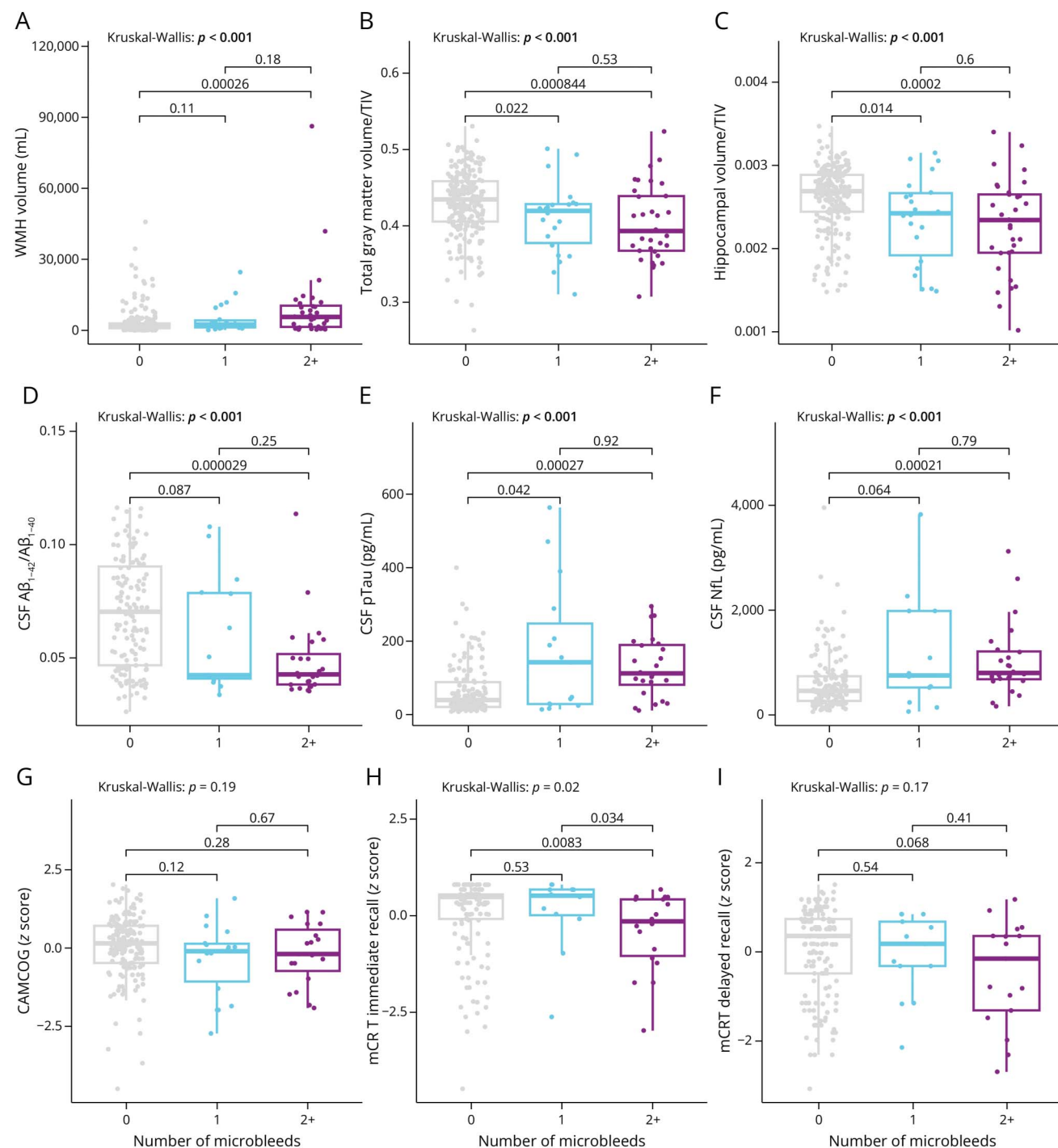
This study analyses microbleeds in a large cohort of individuals with DS. We explored the number and topography of microbleeds, and associations with demographic, clinical, genetic, fluid biomarkers, neuroimaging, and cognitive outcomes. Microbleeds were more prevalent in participants with DS, rose steadily with age starting from 40 years old, and with clinical stage, and were increased in individuals with DS who were *APOE* ϵ 4 allele carriers. They predominated in the cerebellum and posterior regions and showed associations with AD fluid and imaging biomarkers, especially with hippocampal volumes and CSF p-tau-181 concentrations. Together, these results offer valuable insight into the natural course of microbleed development in individuals with a genetically determined form of AD caused by early and rapid amyloid accumulation.

Individuals with DS had a higher microbleed number than controls, and number and severity in participants with DS increased considerably with age and AD clinical stage. Age can be considered as an index of AD disease severity because virtually all individuals with DS are genetically predetermined to develop early onset AD¹ (mean symptom onset = 53.8 years).²⁶ Previous studies in cohorts with DS

have indicated increased microbleeds with an advanced AD clinical stage.^{4,9} The number of microbleeds in controls and aDS was similar despite age differences in these 2 populations, while the largest increase in microbleeds was from aDS to pDS, which is in line with previous findings in individuals with DS.⁹ In autosomal dominant and sporadic AD, microbleeds also increased with age and clinical stage,^{4,24,27} with a gradient of increased microbleeds from controls to mild cognitive impairment, and a further increase for individuals with dementia.

In participants with DS, the *APOE* ϵ 4 allele carriers had a higher number of microbleeds. This is in line with findings from a meta-analysis in the general population, showing that individuals with at least 1 allele are 1.34 times more likely to develop microbleeds.²⁸ The *APOE* ϵ 4 allele has previously been associated in mice with an increased fragility of the cerebral small vessels, resulting in leakage of blood into the parenchyma.²⁹ Similarly, in humans, *APOE* ϵ 4 has been associated with brain-blood barrier breakdown, independent of amyloid.³⁰ In individuals with DS, *APOE* ϵ 4 has been related to earlier symptom onset, age at death, and AD biomarker abnormalities.³¹ In the context of anti-amyloid antibody treatment trials, individuals with *APOE* ϵ 4 alleles demonstrated a higher risk of ARIA-H, likely due to the implication of *APOE* ϵ 4 in accumulation of fibrillar A β in the cerebral vasculature.³ Understanding whether the observed effect of *APOE* ϵ 4 on microbleeds is mediated by AD pathologic processes and/or brain-blood barrier breakdown is, therefore, crucial for risk assessment for treatment trials.

Figure 3 Effect of Microbleed Severity on Imaging and Fluid Biomarkers of Alzheimer Disease, and Cognitive Performance in Individuals With Down Syndrome

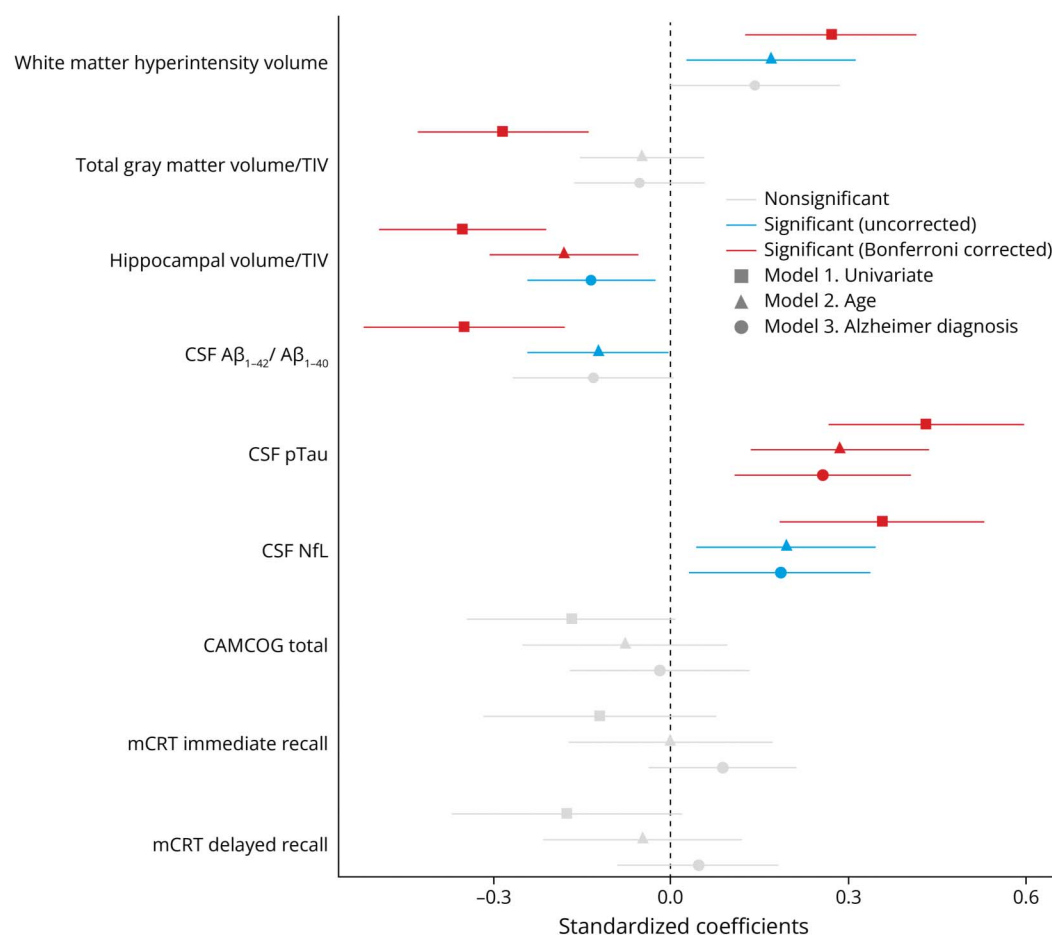


Boxplots representing the effect of microbleed severity (0, 1, 2+) on (A–C) neuroimaging measures, (D–F) Alzheimer disease CSF biomarkers, and (G–I) cognitive test scores. The significance of the group effect was determined using the Kruskal-Wallis test, and pairwise group comparisons were performed using the Mann-Whitney test. Cognitive tests included cognitive performances only for individuals with Down syndrome (DS) who had mild or moderate intellectual disability, were represented as Z-within scores adjusted for intellectual disability. *p*-values surviving Bonferroni correction ($p < 0.01$) are in bold. CAMCOG = Cambridge Cognitive Examination (modified for DS); mCRT = cued recall test (modified for DS); NfL = neurofilament light; PTau = phosphorylated tau; TIV = total intracranial volume; WMH = white matter hyperintensities.

No significant differences in severity or number of microbleeds were observed in association with sex. This is consistent with previous observations in individuals with DS⁹ and autosomal dominant AD.²⁷ This finding also adds to work in

individuals with DS indicating similar clinical progression and biomarkers of AD in biological men and women.³² By contrast, research in the general population reported significant association of male sex on microbleeds.³³ This discrepancy

Figure 4 Regression Models of the Effect of Microbleed Status on Biomarkers and Cognition



Forest plot representing the standardized coefficient and confidence intervals for models of the effect of microbleed status (microbleed+/microbleed-) on (A–C) neuroimaging measures, (D–F) AD CSF biomarkers, (G–I) cognitive test scores (model 1), and when regressing out age (model 2) and Alzheimer diagnosis (model 3). Colors indicate results that survived Bonferroni correction for multiple comparisons (red, $\alpha = 0.05$, $p < 0.00556$, 9 models considered) or an uncorrected threshold of $p < 0.05$ (blue). CAMCOG = Cambridge Cognitive Examination (modified for Down syndrome); mCRT = cued recall test (modified for Down syndrome); NfL = neurofilament light; PTau = phosphorylated tau; TIV = total intracranial volume.

may be explained by distinct sex-dependent lifestyle risk factors in individuals with DS vs the general population, which may become more pronounced at older ages rarely reached by adults with DS. For instance, in the general population, before the age of 60, cardiovascular and cerebrovascular risk factors are more prevalent in men, but after 60, women tend to have an equal or even higher risk.³⁴

Similarly, age-related VRFs—hypertension, diabetes mellitus, and dyslipidemia—had no significant effect on microbleeds. Microbleeds in the general population are mainly attributed to hypertension³⁵ and occur in a nonstrictly lobar pattern^{6,36} (i.e., with at least 1 deep microbleed), while typical age-related VRFs are not prevalent in individuals with DS,^{1,37} which may explain the absence of an association. Only a few deep microbleeds were found in the DS cohort (3.63%, $n = 2$), in individuals without hypertension. Development of microbleeds in this population is therefore not likely to be driven by these VRFs.

Assessment of the topography of microbleeds revealed prevalent lesions throughout the cerebellum (33.66%), temporal (21.29%), and occipital (14.85%) lobes. One criterion for in vivo diagnosis of CAA is a strictly lobar pattern of microbleeds.³⁵ Most microbleeds were lobar; only 2 participants (3.63%) had deep microbleeds. This pattern, which aligns with previous investigations,^{4,9} might therefore be primarily driven by CAA. CAA is highly prevalent in individuals with DS but also in sporadic AD. Postmortem studies indicate a number of 87.10%⁵ and up to 80%,^{5,38} respectively. In sporadic AD, a CAA pattern of lobar microbleeds is also detected.³⁶ Predominantly posterior microbleeds have been detected in individuals with probable CAA,³⁹ and involvement of posterior regions is also observed for other CAA-related lesions, such as WMHs, in different forms of AD including DS,^{9,40} autosomal dominant AD,⁴¹ and sporadic AD.⁴² Hence, our results fit with the common co-occurrence of CAA and AD processes, both of which stem from overproduction and disrupted clearance of Aβ.^{3,8}

Of interest, the cerebellum was quite affected in participants with DS, with 33.66% of microbleeds appearing in this area, or 23 of the 55 individuals with microbleeds. Although 2 cases made up >50% of the cerebellar microbleeds, the cerebellum remained quite affected when removing these cases. In a study with 138 individuals with DS, 17 microbleeds were detected in 15 individuals and 2 individuals (13.33%) had a cerebellar microbleed.⁹ Therefore, cerebellar microbleeds may be slightly more frequent in the DS population. The high number of cerebellar microbleeds has been found in atherosclerotic cerebrovascular disease⁴³ and in CAA.⁴⁴ Since atherosclerosis is rare in individuals with DS,^{1,37} cerebellar microbleeds are thus more likely to be related to CAA in this population.

We also investigated the relationship between microbleeds and imaging and fluid biomarkers, and cognitive outcomes, and found initial significant differences by microbleed severity for all factors. This corroborates previous research in sporadic AD and controls, indicating decreased A β 40 and A β 42,⁴⁵ and increased p-tau-181³⁶ and NfL concentrations⁴⁵ with increased microbleeds. Post hoc tests revealed that differences were mainly driven by the group with 2+ microbleeds (i.e., the group with only 1 microbleed did not differ significantly from the group with no microbleeds except for CSF p-tau-181, $p < 0.05$). This specific effect of severity indicates that the presence of multiple microbleeds has a stronger association with biomarkers of AD than the presence of only 1 microbleed. In fact, previous research suggested that individuals with AD and multiple microbleeds have worse cognitive outcomes, and a higher risk of further microbleeds and mortality.⁶

Since microbleeds increase with AD clinical stage, we performed sensitivity analyses to evaluate whether the relationship between microbleeds and AD biomarkers remained significant, once we controlled for disease severity. We repeated between-group comparisons using a group of microbleed– individuals matched by age, sex, and ID to microbleed+ (eFigure 3). For all comparisons, group differences were no longer significant. Since this approach is stringent and reduces the statistical power, we also performed multivariate models including indices of disease severity (age and clinical stage) as covariates. Only hippocampal volumes and CSF p-tau-181 concentrations remained significantly associated with microbleeds in these models. In previous research, lobar microbleeds were associated with higher CSF p-tau-181 concentrations in a population along the AD continuum *after* adjusting for CSF amyloid β levels and diagnostic group.⁴⁶ According to the authors, 3 hypotheses may explain this finding. First, microbleeds often reflect damaged vascular walls due to increased A β in the vessels, and post mortem studies have demonstrated p-tau aggregation around vessels with increased A β . Second, microbleeds may induce inflammation which leads to tau release into the extracellular space and subsequent elevation of CSF p-tau. Third, individuals with lobar microbleeds, which likely reflect CAA, might be at a more advanced clinical stage, where elevated p-tau is already seen, while CSF A β tends to become abnormal early on in the disease and remains stable later on.⁴⁷ This latter

interpretation might also explain the association with hippocampal volume, which remained after regressing out age.

Finally, we did not find a clear effect of microbleeds on cognitive performance. There was a trend in the univariate models that did not survive when we included disease severity. Previous results on microbleed associations with cognitive functioning have been mixed. Some reports have demonstrated ‘silent’ microbleeds,⁴² and a recent systematic review indicated that microbleeds are not significantly associated with risk of dementia.²⁵ However, other reports have found an effect of microbleeds on risk of dementia⁴⁸ and worse performance on cognitive tests with increased microbleeds, particularly when multiple microbleeds are present.³⁶ If microbleeds do affect cognition in the DS population, they may be too subtle to be detected in comparison with the overall cognitive decline driven by AD pathology. Future studies using larger samples and exploring different cognitive domains are required to shed light on the exact effect of microbleeds on cognition.

The growing recognition of AD in individuals with DS, as well its detrimental effect on life expectancy,²⁶ highlights the ethical importance of considering this population for treatment of approved anti-amyloid agents and future clinical trials.⁴⁹ One observed adverse effect in trials is ARIA-H, which seem to be similar to microbleeds.⁵⁰ ARIA-H are considered to be caused by increased movement and buildup of A β in the cerebral vessels during clearance, resulting in loss of vascular wall integrity and subsequent leakage of blood into the parenchyma.⁸ Recent clinical trials have therefore excluded individuals with ≥ 4 cerebral microbleeds in any location at screening, due to their increased likelihood of subsequent ARIA-H.⁵⁰ Within our cohort, these criteria would exclude 6% of the individuals with DS, including 7% of individuals at the prodromal stage and 12% at the dementia stage. Recent estimates have indicated that 23% of individuals with sporadic AD have microbleeds, and approximately half of these present with multiple microbleeds.⁶ In a population-based cohort of individuals with MCI or dementia, as many as 8.04% of individuals presented with ≥ 4 microbleeds.⁵¹ Thus, the proportion of cases excluded based on the microbleed count would be sensibly similar in individuals with DS and sporadic AD. Therefore, it will be critical to assess whether the same criteria should be used to prevent severe ARIA in individuals with DS, and whether specific topographic parameters of microbleeds (anterior vs posterior, deep vs lobar) can offer valuable insights.

There are some limitations that merit consideration. First, despite rigorous protocols, reliance on visual rating introduces inherent subjectivity in microbleed assessment, emphasizing the need for further development of automated tools, and their validation in individuals with DS. Second, data were acquired on distinct scanners, using different protocols, which could affect the ability to detect microbleeds; however, supplementary analyses indicated no effect of scanner on the results (eFigure 4), and we used high-resolution SWI sequences highly sensitive to detecting microbleeds. Third, although our study included a large cohort of individuals

with DS, the limited number of microbleeds affected our statistical power and ability to find significant associations. Fourth, despite efforts to account for age and AD clinical stage, the inherent covariance between AD pathologic processes in individuals with DS may hinder our capacity to tease apart the effect of different components of the disease. Finally, histopathologic examination was not available, and assessment of neuropathologic substrates of microbleeds in the population of individuals with DS is required to confirm the role of CAA.

In summary, this study better characterizes the contributions and course of microbleeds in a unique population with a predictable AD trajectory. Microbleeds increased in adults with DS with age, AD clinical stage, and higher CSF p-tau-181 concentrations. Despite their increased number in this population, we did not evidence a clear effect on neurodegeneration and cognition. Microbleeds are mainly lobar and predominate in posterior regions and the cerebellum, a pattern suggestive of CAA. Additional studies will provide further clarity on the relationship between AD, CAA, and microbleeds in individuals with DS.

Acknowledgment

The authors thank all the participants with Down’s syndrome, their families, and their carers from the DABNI and SPIN cohorts for their support of, and dedication to this research. We also acknowledge the Fundació Catalana Síndrome de Down (fcsd.org/) for their global support.

Study Funding

This study has been funded by Instituto de Salud Carlos III (ISCIII) through the projects PI18/00435, PI22/00611, INT19/00016, INT23/00048 to D.A.; PI17/01896, AC19/00103 to A.L.; PI14/01126, PI17/01019, PI20/01473 to J.F.; PI18/00335, PI22/00785, ICI23/00032 to M.C.I.; PI22/00307 to A.B., and co-funded by the European Union. This work was also supported by the CIBERNED program (Program 1, Alzheimer’s Disease), the NIH, (NIH grants 1R01AG056850-01A1, 3RF1AG056850-01S1, AG056850, R21AG056974, and R01AG061566 to J.F.), by Generalitat de Catalunya (SLT006/17/125 to DA, SLT002/16/408 to A.L., SLT006/17/119 to J.F.), Generalitat de Catalunya Fundació Tatiana Pérez de Guzmán el Bueno (IIBSP-DOW-2020-151 to J.F.), ‘MES-CoBraD’ (H2020-SC1-BHC-2018-2020/GA 965422 to J.F.), and “Marató TV3” foundation grants (20142610 to A.L. and 20141210 to J.F.). The manuscript describes independent research, and the views expressed are those of the authors and not necessarily those of the funders. The sponsors of the study did not take part in the design and conduct of the study; collection, management, analysis, and interpretation of the data; writing and review of the report; or the decision to submit the article for publication.

Disclosure

M.R. Aranha has provided paid consultancy for Veranex, and is a partner and director of production at Masima—Soluções em Imagens Médicas LTDA. J.E. Arriola-Infante has received research support from the Río Hortega Fellowship from Carlos III Health Institute (CM21/00243). V. Montal has

received support from a predoctoral grant from the Instituto de Salud Carlos III (FI18/00275). J. Arranz has received research support from the Río Hortega Fellowship from Carlos III Health Institute (CM22/00219). D. Alcolea has participated in advisory boards from Fujirebio-Europe, Roche Diagnostics, Grifols S.A., and Lilly, has received speaker honoraria from Fujirebio-Europe, Roche Diagnostics, Nutricia, Krka Farmacéutica S.L., Zambon S.A.U., and Esteve Pharmaceuticals S.A, and reports a filed patent application (WO2019175379 A1 Markers of synaptopathy in neurodegenerative disease, licensed to ADx, EPI8382175.0). A. Lleó has participated in advisory boards from Biogen, Eisai, Fujirebio-Europe, Grifols, Novartis, Roche, Otsuka Pharmaceutical, Nutricia, Zambón, and NovoNordisk, and reports a filed patent application (WO2019175379 A1 Markers of synaptopathy in neurodegenerative disease, licensed to ADx, EPI8382175.0). J. Fortea has served on advisory boards, served on adjudication committees, or received speaker honoraria from Roche, NovoNordisk, Esteve, Biogen, Laboratorios Carnot, Adamed, LMI, Novartis, Lundbeck, Roche, AC Immune, Alzheimer, Zambon, Lilly, Spanish Neurological Society, T21 Research Society, Lumind Foundation, Jérôme Lejeune Foundation, Alzheimer’s Association, NIH, USA, and Instituto de Salud Carlos III, and reports a filed patent application (WO2019175379 A1 Markers of synaptopathy in neurodegenerative disease, licensed to ADx, EPI8382175.0). M. Carmona-Iragui has received funding for grants from Alzheimer’s Association (AARG-22-973966 and GBHI_ALZ-18-543740 together with Global Brain Health Institute), Jérôme Lejeune Foundation (Project 1913, Cycle 2019B), and Societat Catalana de Neurologia (SCN 2020); has participated in an advisory board from Roche, and has received speaker honoraria from Neuraxpharm. A. Bejanin acknowledges support from a Miguel Servet grant from the Institute of Health Carlos III (CP20/00038) and the Alzheimer’s Association (AARG-22-923680). The other authors report no relevant disclosures. Go to [Neurology.org/N](https://www.neurology.org/N) for full disclosures.

Publication History

Received by *Neurology* March 1, 2024. Accepted in final form May 31, 2024. Submitted and externally peer reviewed. The handling editor was Associate Editor Linda Hershey, MD, PhD, FAAN.

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Continued

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Appendix *(continued)*

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