Comparison of CSF biomarkers in Down syndrome and autosomal dominant Alzheimer's disease: a cross-sectional study

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Summary

Background Due to trisomy of chromosome 21 and the resultant extra copy of the amyloid precursor protein gene, nearly all adults with Down syndrome develop Alzheimer's disease pathology by the age of 40 years and are at high risk for dementia given their increased life expectancy compared with adults with Down syndrome in the past. We aimed to compare CSF biomarker patterns in Down syndrome with those of carriers of autosomal dominant Alzheimer's disease mutations to enhance our understanding of disease mechanisms in these two genetic groups at high risk for Alzheimer's disease.

Methods We did a cross-sectional study using data from adults enrolled in the Alzheimer's Biomarker Consortium-Down Syndrome (ABC-DS) study, a multisite longitudinal study of Alzheimer's disease in Down syndrome, as well as a cohort of carriers of autosomal dominant Alzheimer's disease mutations and non-carrier sibling controls enrolled in the Dominantly Inherited Alzheimer Network (DIAN) study. For ABC-DS, participants with baseline CSF, available clinical diagnosis, and apolipoprotein E genotype as of Jan 31, 2019, were included in the analysis. DIAN participants with baseline CSF, available clinical diagnosis, and apolipoprotein E genotype as of June 30, 2018, were evaluated as comparator groups. CSF samples obtained from adults with Down syndrome, similarly aged carriers of autosomal dominant Alzheimer's disease mutations, and non-carrier siblings (aged 30–61 years) were analysed for markers of amyloid β ($A\beta_{1–40}$, $A\beta_{1–42}$); tau phosphorylated at threonine 181-related processes; neuronal, axonal, or synaptic injury (total tau, visinin-like protein 1, neurofilament light chain [NfL], synaptosomal-associated protein 25); and astrogliosis and neuroinflammation (chitinase-3-like protein 1 [YKL-40]) via immunoassay. Biomarker concentrations were compared as a function of dementia status (asymptomatic or symptomatic), and linear regression was used to evaluate and compare the relationship between biomarker concentrations and age among groups.

Findings We assessed CSF samples from 341 individuals (178 [52%] women, 163 [48%] men, aged 30–61 years). Participants were adults with Down syndrome (n=41), similarly aged carriers of autosomal dominant Alzheimer's disease mutations (n=192), and non-carrier siblings (n=108). Individuals with Down syndrome had patterns of Alzheimer's disease-related CSF biomarkers remarkably similar to carriers of autosomal dominant Alzheimer's disease mutations, including reductions (all $p<0·0080$) in $A\beta_{1–42}$ to $A\beta_{1–40}$ ratio and increases in markers of phosphorylated tau-related processes; neuronal, axonal, and synaptic injury ($p<0·080$); and astrogliosis and neuroinflammation, with greater degrees of abnormality in individuals with dementia. Differences included overall higher concentrations of Aβ and YKL-40 (both $p<0·0008$) in Down syndrome and potential elevations in CSF tau ($p<0·010$) and NfL ($p<0·0001$) in the asymptomatic stage (ie, no dementia symptoms).

Interpretation CSF biomarker profiles are useful for identifying and tracking Alzheimer's disease-related processes in Down syndrome and, as such, are likely to have use in clinical trial design in this understudied population at risk.

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Introduction

Due to trisomy of chromosome 21 and the resultant extra copy of the amyloid precursor protein (*APP*) gene, nearly all adults with Down syndrome will develop amyloid and tau pathology consistent with Alzheimer's disease by the age of 40 years.[1] Risk of Alzheimer's disease dementia in this population is age-dependent, with estimates of around 50% prevalence by 50 years and around 90% by 70 years.[2] However, dementia has a heterogeneous presentation in Down syndrome, including age of onset

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Research in context

Evidence before this study

We searched PubMed regularly for all relevant English-language articles relating to CSF biomarkers of Alzheimer's disease in individuals with Down syndrome or autosomal dominant Alzheimer's disease published from database inception until May 1, 2020, for consideration of inclusion in this report. Search terms included "Alzheimer disease", "autosomal dominant Alzheimer disease", "biomarker", "brain", "cerebrospinal fluid", and "Down syndrome". Few studies evaluating fluid biomarkers of Alzheimer's disease in individuals with Down syndrome have been published, whereas the biomarker profiles of individuals from families with autosomal dominant Alzheimer's disease enrolled in the Dominantly Inherited Alzheimer Network (DIAN) study have been well characterised; however, we found no direct comparisons of biomarker profiles between individuals with Down syndrome and those from families with known autosomal dominant Alzheimer's disease mutations, the two genetically determined at-risk groups for developing Alzheimer's disease.

Added value of this study

To our knowledge, this is the first study directly comparing CSF biomarkers of Alzheimer's disease between individuals with autosomal dominant Alzheimer's disease and adults with Down syndrome. There are substantial similarities in the profile of CSF biomarkers in adults with Down syndrome and those in individuals with autosomal dominant Alzheimer's disease. However, variations in some markers could shed light on potential differences in amyloid β metabolism, neuronal injury, and astrogliosis and neuroinflammation in the setting of Down syndrome.

Implications of all the available evidence

Our results support the use of CSF biomarker profiles for identifying and tracking Alzheimer's disease-related processes in Down syndrome and, as such, are likely to be useful for clinical trial design in this understudied at-risk population. However, the overall higher amounts of amyloid β and potential preclinical (presymptomatic) elevations in markers of neuronal injury and astrogliosis as well as neuroinflammation in Down syndrome highlight inherent metabolic differences in the setting of trisomy 21. These differences should be considered when defining CSF cutoff values for identification of underlying Alzheimer's disease pathologies, which might be required for clinical trial enrolment and evaluation of target engagement and biomarker outcomes. In-depth investigation of longitudinal change in biomarkers across the disease spectrum in cohorts of adults with Down syndrome is still needed to fully characterise the biomarker profiles and the appropriate age and time for intervention.

and clinical symptoms. Furthermore, the time course of disease progression in adults with Down syndrome remains uncertain.

Alzheimer's disease-related biomarkers have informed our understanding of pathological disease progression in individuals at risk for developing late-onset Alzheimer's disease[3] and in individuals carrying autosomal dominant Alzheimer's disease mutations, given the near 100% penetrance of mutations and the reliable expected age at symptomatic onset within affected families. Although carriers of autosomal dominant Alzheimer's disease mutations develop dementia when aged around 30–60 years,[4] biomarker changes are detectable 20–30 years before symptom onset.[5] This finding provides support for the existence of a long, asymptomatic stage during which disease-modifying interventions might be most effective and provides a framework to compare other at-risk Alzheimer's disease cohorts such as adults with Down syndrome.

Biomarkers of Alzheimer's disease pathology (amyloid via CSF amyloid β 1–42 [$A\beta_{1-42}$] and PET; phosphorylated tau-related processes via CSF tau phosphorylated at threonine 181 [p-tau181] and tau PET; neuronal injury via CSF total tau and neurofilament light chain [NfL] and regional brain atrophy via MRI) have been reported in studies of Down syndrome.[2,6–8] However, cohorts have typically been small, and comparator groups (if any) are mostly older, hampering characterisation of pathological disease progression and correlation with clinical status.

To address these limitations, CSF biomarker profiles in a cohort of adults with Down syndrome were compared with those from autosomal dominant Alzheimer's disease families (both with and without a dementia diagnosis). Both populations have genetic causes of Alzheimer's disease (triplication of *APP* in Down syndrome and mutations in *APP*, presenilin 1 [*PSEN1*], or presenilin 2 [*PSEN2*] in autosomal dominant Alzheimer's disease) that drive overproduction of Aβ ($A\beta_{1-42}$ in autosomal dominant Alzheimer's disease and total Aβ in Down syndrome) and thus share a potential common disease cause. These groups at risk also develop Alzheimer's disease at similar ages, with risk increasing with advancing age, allowing age-similar comparisons to be made between individuals with Down syndrome and those with autosomal dominant Alzheimer's disease mutations, and between the genetic groups and autosomal dominant Alzheimer's disease non-carrier sibling controls. This comparison allowed examination of age-related biomarker patterns among the three groups (Down syndrome, non-carriers, mutation carriers) using cross-sectional data. Although the metric of estimated years to symptom onset can be used in autosomal dominant Alzheimer's disease due to the relatively consistent age of onset within families, such a metric does not exist in Down syndrome. We hypothesised that CSF

biomarker profiles would be similar between the groups at risk, with both differing from the non-carrier controls.

We aimed to analyse CSF for markers of amyloid, phosphorylated tau-related processes, neuronal or axonal injury, synaptic dysfunction, and astrogliosis and neuroinflammation. Study of groups at risk not only affords the opportunity to understand the timing and sequence of pathological changes associated with Alzheimer's disease, but direct comparison could also shed light on possible differences in Aβ metabolism, neuronal injury, or neuroinflammation in the setting of trisomy 21 compared with Alzheimer's disease-causing mutations. Knowledge from this novel comparison might be useful for informing clinical trial design in these understudied groups at risk.

Methods

Study design and participants

We did a cross sectional study. Adults with Down syndrome were enrolled in the Alzheimer's Biomarker Consortium-Down Syndrome (ABC-DS) study. Participants with baseline CSF (and available clinical diagnosis and apolipoprotein E [*APOE*] genotype) enrolled in ABC-DS between Jan 27, 2015, and Dec 18, 2018, were included in the analyses. All participants meeting these criteria were aged between 30–61 years. The ABC-DS cohort included participants from four performance sites in the USA. ABC-DS is a longitudinal study of Alzheimer's disease in Down syndrome incorporating neuropsychological, neuroimaging, genetic, and fluid biomarker measures.[9] Biomarker data from an overlapping ABC-DS cohort were published in 2020,[10] but without comparison to non-Down syndrome controls or other Alzheimer's disease cohorts, notably those due to genetic causes.

To avoid potential age-related bias, CSF samples from a cohort of carriers of autosomal dominant Alzheimer's disease mutations and non-carrier sibling controls enrolled in the Dominantly Inherited Alzheimer Network (DIAN) study[11] (from Jan 26, 2009, to June 30, 2018) within the same age range (30–61 years) were chosen as ABC-DS comparator groups. The DIAN cohort included participants from 18 performance sites across six countries (Argentina, Australia, Germany, Japan, the UK, and the USA).

Participants with the Dutch mutation (*APP* Glu693Gln mutation) were excluded because they manifest an atypical clinical syndrome.[12] Informed consent was obtained directly from all participants whenever possible; otherwise, assent was obtained, and informed consent obtained from the participant's legally authorised representative. Institutional review board approval was obtained at all sites.

Procedures

The ABC-DS uses neuropsychological measures with the strongest evidence for defining different stages of dementia, most of which were developed specifically for Down syndrome[9] (appendix p 2). Based on cognitive testing, assessments of neurological and overall health status, as well as caregiver-provided information on individual health history, adaptive functioning, and possible symptoms of Alzheimer's disease (without regard to biomarkers), participants received a diagnosis of cognitively stable; mild cognitive impairment; possible, probable, or definite dementia (Alzheimer's disease); or uncertain (due to complications unrelated to Alzheimer's disease), using a consensus-based protocol. This protocol takes the level of pre-existing intellectual disability into consideration. A diagnosis of cognitively stable indicated performance consistent with past intellectual functioning and current age. Mild cognitive impairment indicated evidence of cognitive decline over time beyond what would be expected with advancing age but of insufficient severity to suggest dementia. Alzheimer's disease indicated clear evidence of substantial cognitive and functional decline with a high degree of confidence in the dementia rating. For the present study, individuals who were cognitively stable were classified as asymptomatic (no dementia), and the combined mild cognitive impairment and Alzheimer's disease group was classified as symptomatic. Participants who received a diagnosis of uncertain were excluded.

Dementia status in DIAN was defined using the clinical dementia rating (CDR) scale (CDR 0=normal cognitive function; 0·5=very mild dementia, 1=mild dementia, 2=moderate dementia, and 3=severe dementia).[13] Standardised assessments ascertained family history of Alzheimer's disease and medical history, and participants underwent comprehensive neurological examination and neuropsychological assessment of general cognitive function, memory, attention, executive function, visuospatial function, and language.[11] Clinicians were masked to mutation status and biomarker data. To enable comparisons with the Down syndrome cohort, CDR 0 in DIAN was defined as asymptomatic (DIAN asymptomatic mutation carriers), and CDR more than 0 was defined as symptomatic (DIAN symptomatic mutation carriers).

Karyotype for ABC-DS participants was obtained from medical records or a designated cytogenetic laboratory. For DIAN participants, DNA sequencing for autosomal dominant Alzheimer's disease mutations (*APP*, *PSEN1,* or *PSEN2*) was done using PCR-based amplification of the appropriate exon followed by Sanger sequencing.

APOE genotype was also established. Two *APOE* single nucleotide polymorphisms (SNPs; rs429358 and rs7412) determined the presence of *APOE* ε2, ε3, and ε4 alleles (ABC-DS via KASP genotyping system by LGC Genomics, Beverly, MA, USA; DIAN via Applied Biosystems' TaqMan assay, Waltham, MA, USA). *APOE* ε4 status was dichotomised as ε4-negative or ε4-positive (comprising both ε4 heterozygotes and homozygotes).

Protocols for CSF collection and processing were consistent with the Alzheimer's Disease Neuroimaging Initiative; notably in terms of use of polypropylene tubes and aliquot size (0·5 mL). Participants in ABC-DS

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See **Online** for appendix

For **ABC-DS study information** see https://www.nia.nih.gov/research/abc-ds

For **DIAN study information** see https://dian.wustl.edu/our-research/observational-study/

For the **Alzheimer's Disease Neuroimaging Initiative** see http://www.adni-info.org/

underwent lumbar puncture at between 1100–1600 h; 10–20 mL of CSF was collected via gravity drip, aspiration, or assisted by fluoroscopy. DIAN participants underwent lumbar puncture at around 0800 h after overnight fasting; 20–30 mL of CSF was collected via gravity drip. CSF from both cohorts was flash frozen on dry ice before shipment to the ABC-DS and DIAN biomarker core laboratory at Washington University (St Louis, MO, USA). Samples were thawed and aliquoted into polypropylene tubes before storage at –80°C. $A\beta_{1-40}$, $A\beta_{1-42}$, total tau, and p-tau181 were measured in batch (second freeze-thaw) via an automated immunoassay (LUMIPULSEG1200, Fujirebio, Malverne, PA, USA). ABC-DS and DIAN samples were each analysed in batch for emerging biomarkers. Synaptosomal-associated protein 25 (SNAP-25) and visinin-like protein 1 (VILIP-1) were measured (second freeze-thaw) using Single Molecule Counting technology (originally developed for the Singulex Erenna System, now part of EMD Millipore, Burlington, MA, USA) using antibodies developed at the Department of Pathology and Immunology at Washington University School of Medicine (St Louis, MO, USA).[5] NfL (UmanDiagnostics, Umeå, Sweden) and chitinase-3-like protein 1 (YKL-40, Quidel, San Diego, CA, USA) were measured (third freeze-thaw) via commercial ELISA according to manufacturer instructions. Kit controls and pooled CSF samples were included to ascertain data reproducibility for defining quality control criteria (eg, assay-specific cutoffs for percentage coefficients of variation [%CV]).

Statistical analysis

Normality assumption of the continuous variables were examined in each group using normal quantile-quantile plots. All continuous variables were approximately normally distributed, except NfL, which was right skewed and was log-transformed. Demographic group differences between DIAN non-carriers, DIAN mutation carriers, and adults with Down syndrome were compared using one-way ANOVA *F* test for continuous variables and χ^2 tests for categorical variables. If significant, post-hoc pairwise comparisons were done using the two sample *t* test for continuous variables and χ^2 test or Fisher's exact test (as appropriate) for categorical variables. Linear regression compared mean biomarker concentrations among the genetic and cognitive groups (DIAN non-carriers, asymptomatic with Down syndrome, symptomatic with Down syndrome, DIAN asymptomatic mutation carriers, DIAN symptomatic mutation carriers) and included age, *APOE* ε4 status (ε4-positive or ε4-negative), sex (because advanced age, *APOE* ε4-positivity, and female sex are known Alzheimer's disease risk factors), and their interactions with group as covariates. Interactions between *APOE* and group and between sex and group were not significant for any biomarkers so were excluded from the final models. Linear regressions were used to compare the biomarker slopes of the three groups (Down syndrome, DIAN non-carrier, and DIAN mutation carrier). Each linear regression included one biomarker as the outcome, and group, age, *APOE* ε4 status, and the interaction between age and group as the predictors. To account for the potential correlation in participants from the same family in DIAN, sensitivity analyses were done using linear mixed effects models with a random intercept for each family cluster. There was no family cluster in Down syndrome, so each participant was treated as a family cluster. As all the participants in the Down syndrome group were white, race was not included as a covariate in the linear regressions, but we did sensitivity analyses based on a subset of white participants. For all analyses, p values for prespecified subgroup comparisons were adjusted by the Benjamini-Hochberg method.[14] Pre-specified subgroup comparisons (as shown in appendix pp 5–7) were determined based on specified research questions. For each linear regression, participants with missing biomarker data were omitted from the model. Analyses used R, version 3.6.2 and SAS, version 9.4.

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

We assessed 41 CSF samples from adults with Down syndrome (26 men and 15 women) enrolled in the parent ABC-DS study between Jan 27, 2015, and Dec 18, 2018, and 300 CSF samples from individuals (137 men and 163 women) enrolled in the parent DIAN study between Jan 26, 2009, and June 30, 2018. DIAN samples included similarly aged (30–61 years) carriers of autosomal dominant Alzheimer's disease mutations (n=192) and non-carrier (n=108) siblings. Demographic data are reported in the table and the appendix (pp 2–4). Karyotyping in Down syndrome revealed 33 (80%) individuals with trisomy 21, two (5%) with mosaicism, and two (5%) with translocation. Four (10%) individuals were missing karyotype information at the time of analysis (appendix p 2). Most DIAN participants were from families with the *PSEN1* mutation (74 [68%] DIAN non-carrier, 143 [75%] DIAN mutation carriers); 15 (14%) DIAN non-carrier and 20 (10%) DIAN mutation carriers were from *PSEN2* families; and 19 (18%) DIAN non-carrier and 29 (15%) DIAN mutation carriers were from *APP* families. Specific participant genotypes are shown in the appendix (p 4). Although age ranges were identical among the groups by design, the mean age of participants with Down syndrome (48·7 years [SD 7·3]) was older than DIAN non-carriers (41·7 years [8·8]) and DIAN mutation carriers (41·3 years [8·3]). The Down syndrome group had a larger percentage of men (63%) than the two autosomal dominant Alzheimer's disease groups (44–46%), although this difference was not significant. Despite each group being predominantly white (>88%), the DIAN mutation carriers group contained a larger percentage of non-white

	DIAN non-carriers group (n=108)	Down syndrome group (n=41)	DIAN mutation carriers group (n=192)	p value
Age, years	41·7 (8·8)	48·7 (7·3)*†	41·3 (8·3)	<0·0010
Sex				
Female	60 (56%)	15 (37%)	103 (54%)	..
Male	48 (44%)	26 (63%)	89 (46%)	0·098
APOE ε4-positive	38 (35%)	16 (39%)	62 (32%)	0·68
Race	..	..	..	0·016
White	100 (93%)	41 (100%)	169 (88%)	..
Non-white	6 (6%)	0	23 (12%)	..
Unknown	2 (2%)	0	0	..
Cognitive status	..	..	..	<0·0010
Asymptomatic	108 (100%)	27 (66%)	110 (57%)	..
Symptomatic	0	14 (34%)	82 (43%)	..
Clinical dementia rating				
0	105 (97%)	NA	110 (57%)	..
0·5	3 (3%)	NA	55 (29%)	..
1	0	NA	20 (10%)	..
2	0	NA	5 (3%)	..
3	0	NA	2 (1%)	..
CSF biomarkers, n				
$A\beta_{1-40}$, pg/mL	9128 (2845)	13 612 (3892)*†	8698 (2810)	<0·0010
$A\beta_{1-42}$, pg/mL	817 (285)	877 (287)†	535 (286)*	<0·0010
Total tau, pg/mL	262 (113)	644 (382), n=39*	554 (362), n=188*	<0·0010
p-tau181, pg/mL	30 (13), n=106	93 (77)*	90 (70), n=189*	<0·0010
$A\beta_{1-42}$ to $A\beta_{1-40}$ ratio	0·09 (0·01)	0·07 (0·02)*	0·06 (0·03)*	<0·0010
Total tau to $A\beta_{1-42}$ ratio	0·35 (0·20)	0·84 (0·62), n=39*†	1·44 (1·27), n=188*	<0·0010
p-tau181 to $A\beta_{1-42}$ ratio	0·04 (0·02), n=106	0·13 (0·14)*†	0·24 (0·24), n=189*	<0·0010
VILIP-1, pg/mL	139 (55), n=82	202 (92)*	184 (83), n=146*	<0·0010
SNAP25, pg/mL	3·9 (1·5), n=82	4·6 (1·8)‡	4·9 (2·0), n=145*	<0·0010
YKL-40, ng/mL	150 (71), n=82	251 (127), n=38*†	187 (83), n=146*	<0·0010
log NfL, pg/mL	2·83 (0·19), n=64	3·24 (0·27)*†	3·03 (0·32), n=109*	<0·0010

Data are mean (SD) or n (%). DIAN participants who had a clinical dementia rating score of 3 at the time of CSF collection are not shown in the table to maintain masking as to mutation status. Missing CSF data reflect samples that were not available at the time of DIAN data freeze analysis (non-carrier: n=26 [SNAP-25, VILIP-1, YKL-40] and n=44 NfL; mutation carriers: n=46 [SNAP-25, VILIP-1, YKL-40] and n=83 NfL), or did not pass quality control criteria (Down syndrome: n=2 tau, n=3 YKL-40; mutation carriers: n=4 tau, n=3 p-tau181, n=1 SNAP-25). Non-white race included Black or African-American, American Indian or Alaska native, Native Hawaiian or other Pacific Islander, and Asian. We cannot specify non-white race due to the small number of participants, which could lead to unmasking. Aβ=amyloid β. *APOE*=apolipoprotein E. NfL=neurofilament light chain. SNAP-25=synaptosomal-associated protein 25. VILIP-1=visinin-like protein 1. YKL-40=chitinase-3-like protein 1. *Significantly different (at least p<0·008) from non-carrier. †Significantly different (at least p<0·0008) from mutation carriers. ‡Non-significant trend (at least p<0·08) from non-carrier. Specific p values are shown in the appendix (p 3).

Table: **Demographic characteristics and CSF biomarker concentrations**

(ie, Black or African American, American Indian or Alaska Native, or Native Hawaiian or Other Pacific Islander Asian) participants than the other two groups. Removal of non-white participants did not change the overall outcome of any analyses. *APOE* ε4 status (around 35% positive) was not different among the groups. Although three of 108 DIAN non-carriers were CDR 0·5, all were classified asymptomatic since they were CDR 0 at follow-up. 14 (34%) participants with Down syndrome (53·2 years [SD 4·5], range 45–61) and 82 (43%) DIAN mutation carriers (45·6 years [8·2], range 30–61) were symptomatic. Of the 14 participants with Down syndrome who were symptomatic, 50% were classified as having mild cognitive impairment. Of the 82 symptomatic DIAN mutation carriers, 67% had very mild dementia (CDR 0·5, similar to the level of impairment in mild cognitive impairment; table).

In general, concentrations of most biomarkers in DIAN mutation carriers and Down syndrome differed from those in the DIAN non-carrier group (table, appendix p 3). DIAN mutation carrier patterns were consistent with the presence of Alzheimer's disease pathology, including reductions in $A\beta_{1-42}$ and $A\beta_{1-42}$ to $A\beta_{1-40}$ ratio (measure of amyloid; both p<0·0001), elevated p-tau181 (measure of phosphorylated tau-related processes; p<0·0001), increases in markers of neuronal or axonal injury (total tau, VILIP-1, NfL; all p<0·0001), and presynaptic dysfunction (SNAP-25; p=0·0001), and elevations in YKL-40 (marker of astrogliosis

and neuroinflammation; p=0·0070) compared with the DIAN non-carrier group. The tau to $A\beta_{1–42}$ and p-tau181 to $A\beta_{1–42}$ ratios were also higher in DIAN mutation carriers versus DIAN non-carriers (both p<0·0001).

Similar findings were observed in Down syndrome versus DIAN non-carriers for all biomarkers except $A\beta_{1–40}$ and $A\beta_{1–42}$. Unlike DIAN mutation carriers, adults with Down syndrome had higher $A\beta_{1–40}$ than DIAN non-carriers (p<0·0001), whereas $A\beta_{1–42}$ concentrations were not different (p=0·49). Some analytes differed between Down syndrome and DIAN mutation carriers; $A\beta_{1–40}$ (p<0·0001), $A\beta_{1–42}$ (p<0·0001), YKL-40 (p=0·0002), and NfL (p=0·0002) were significantly higher in Down syndrome than in DIAN mutation carriers, whereas total tau to $A\beta_{1–42}$ ratio (p=0·0017) and p-Tau181 to $A\beta_{1–42}$ ratio (p=0·0023) were lower, likely due to overall higher $A\beta_{1–42}$ in Down syndrome. Exploratory analyses in the subset of *APP* DIAN mutation carriers (n=29) revealed similar, but not identical, patterns compared with individuals with mutations in *PSEN1* and *PSEN2* (appendix pp 2, 5).

As biomarker profiles are known to change with increasing disease severity, we next compared the groups as a function of dementia status, performing analyses separately for the DIAN mutation carriers and Down syndrome groups. 27 (66%) individuals with Down syndrome were asymptomatic (cognitively stable) and 14 (34%) were symptomatic. 110 (57%) participants in the DIAN mutation carrier group were asymptomatic and 82 (43%) were symptomatic (table). Although $A\beta_{1–40}$ was higher in Down syndrome than in the DIAN mutation carrier group (table), concentrations did not differ with dementia status in those in the DIAN mutation carrier group (p=0·14; figure 1A), but were different as a function of dementia status in the Down syndrome group (p=0·040). By contrast, $A\beta_{1–42}$ concentrations were lower in individuals who were symptomatic in both groups (p=0·0010 for Down syndrome; p<0·0001 for DIAN mutation carriers; figure 1B), as was the $A\beta_{1–42}$ to $A\beta_{1–40}$ ratio in DIAN mutation carriers (p<0·0001) but not the Down syndrome group (p=0·14; figure 1C). p-tau181 was markedly higher in individuals who were symptomatic in both groups (p=0·0004 for Down syndrome; p<0·0001 for DIAN mutation carriers; figure 1E). Although total tau was higher in those in the DIAN symptomatic mutation carrier group versus those in the DIAN asymptomatic mutation carrier group (p<0·0001), it was not elevated in symptomatic Down syndrome versus asymptomatic Down syndrome (p=0·17). This muted symptom-related elevation in total tau in Down syndrome (mean difference of 137 pg/mL in Down syndrome versus 358 pg/mL in DIAN mutation carriers) is likely to reflect the high amounts already apparent in those who were asymptomatic (asymptomatic Down syndrome greater than DIAN asymptomatic mutation carriers; p=0·0020; figure 1D). However, the higher mean age of the Down syndrome group likely contributed to this effect as significance was lost after adjusting for age, *APOE* ε4 status, and sex (appendix pp 6–7). Although amounts of YKL-40 were higher in individuals who were symptomatic versus asymptomatic in both groups (p=0·010 for Down syndrome; p<0·0001 for DIAN mutation carriers), they were also higher overall in the Down syndrome group versus the DIAN mutation carrier group (p=0·0003 for asymptomatic; p=0·010 for symptomatic; figure 1F), although statistical significance was lost after adjusting for covariates (appendix pp 6–7). Down syndrome biomarker concentrations as a function of karyotype are shown in the appendix (p 9). Small numbers of non-trisomy 21 cases precluded statistical analysis.

Overall the total tau to $A\beta_{1–42}$ and p-Tau181 to $A\beta_{1–42}$ ratios were higher in symptomatic versus asymptomatic groups (figure 1G, H), but total tau to $A\beta_{1–42}$ in Down syndrome did not reach statistical significance (Down syndrome: total tau to $A\beta_{1–42}$ p=0·11, p-tau181 to $A\beta_{1–42}$ p=0·010; DIAN mutation carriers: total tau to $A\beta_{1–42}$ and p-tau181 to $A\beta_{1–42}$, both p<0·0001). NfL was higher in symptomatic versus asymptomatic groups (p=0·0010 for Down syndrome; p<0·0001 for DIAN mutation carriers; figure 1I), with elevations already apparent in asymptomatic Down syndrome (p<0·0001 for asymptomatic Down syndrome *vs* DIAN asymptomatic mutation carriers), although significance was lost after adjusting for covariates (appendix pp 6–7). Individuals who were symptomatic in the DIAN mutation carrier group had elevations in VILIP-1 (p=0·0001; figure 1F) and SNAP-25 (p<0·0001; figure 1K) compared with those who were asymptomatic in the DIAN mutation carrier group, whereas differences did not reach statistical significance in those with Down syndrome (VILIP-1 p=0·060; SNAP-25 p=0·30).

We next modelled biomarker patterns over the course of disease progression by comparing concentrations as a function of age. Although mutation carriers in families with different autosomal dominant Alzheimer's disease mutations develop dementia at different ages, disease pathology increases with advancing age,[4] as it does in Down syndrome.[8] Slope comparisons showed decreases in $A\beta_{1–42}$ (p=0·0001) and $A\beta_{1–42}$ to $A\beta_{1–40}$ ratio (p=0·0030; figure 2B, C) and increases in total tau (p=0·0006; figure 2D), p-tau181 (p=0·0020; figure 2E), total tau to $A\beta_{1–42}$ and p-tau181 to $A\beta_{1–42}$ ratios (both p<0·0001; figure 2G, H), and SNAP-25 (p=0·030; figure 2K) in the DIAN mutation carrier versus DIAN non-carrier groups with advancing age, consistent with pathological disease progression over time. Although markers of astrogliosis and neuroinflammation (YKL-40, figure 2F) and neuronal or synaptic injury (NfL, figure 2I; VILIP-1, figure 2J) in those in the DIAN mutation carrier group also increased with age, their slopes were not different from DIAN non-carrier controls.

Age-related biomarker patterns in the Down syndrome and DIAN mutation carrier groups were remarkably similar (figure 2, appendix p 8), although fewer markers in Down syndrome significantly differed from the DIAN non-carrier group, and some differences between the

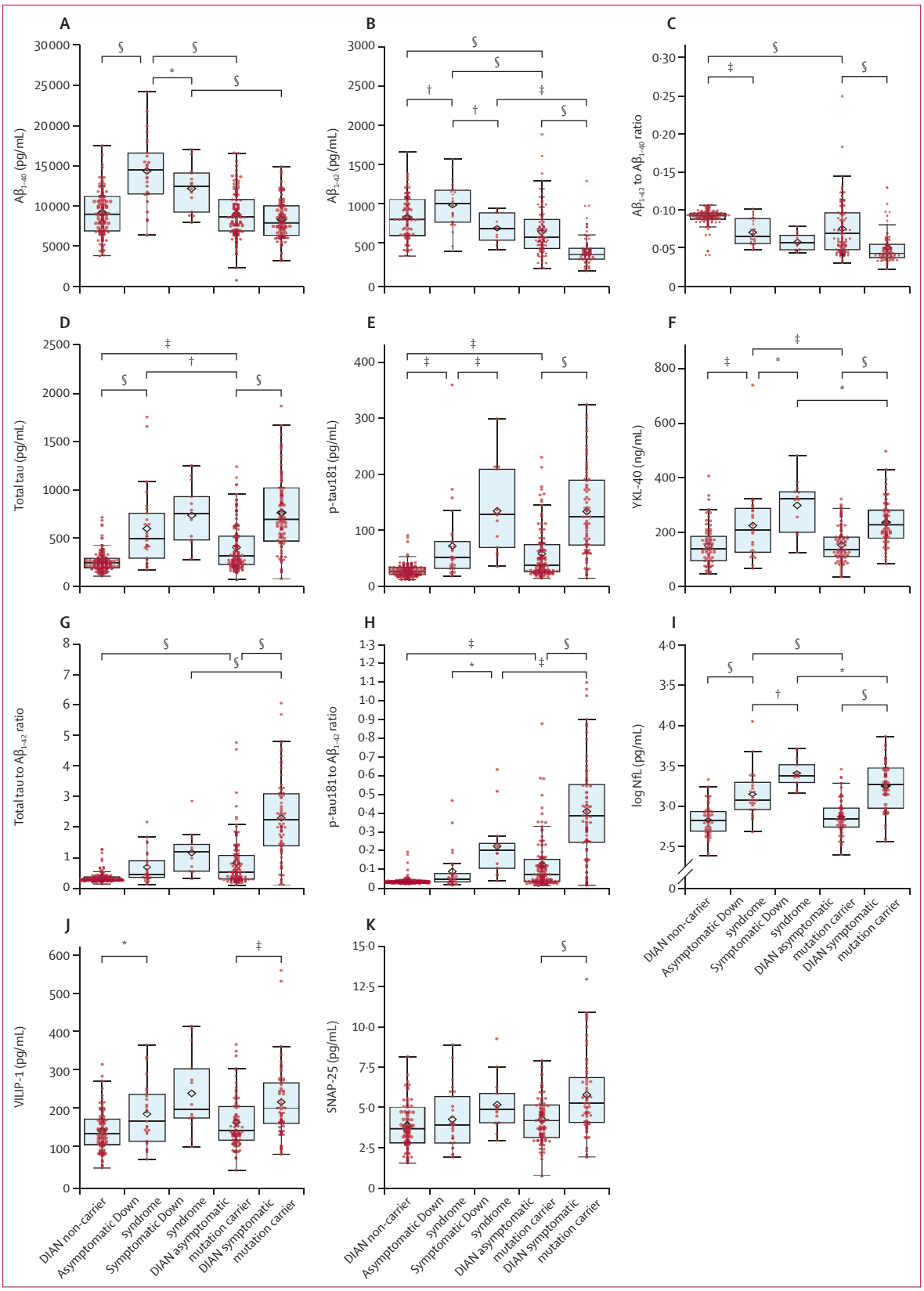

Figure 1: **CSF biomarkers of amyloid, tau, and phosphorylated tau-related processes, astrogliosis and neuroinflammation, and neuronal, synaptic, and axonal injury**
Biomarkers included (A) $Aβ_{1-40}$, (B) $Aβ_{1-42}$, (C) $Aβ_{1-42}$ to $Aβ_{1-40}$ ratio, (D) total tau, (E) p-tau181, (F) YKL-40, (G) total tau to $Aβ_{1-42}$ ratio, (H) p-tau181 to $Aβ_{1-42}$ ratio, (I) log transformed NfL, (J) VILIP-1, and (K) SNAP25. The central horizontal bar shows the median value, and the lower and upper boundaries show the IQR. The diamond indicates the group mean. DIAN=Dominantly Inherited Alzheimer Network. NfL=neurofilament light chain. p-tau181=tau phosphorylated at threonine 181. SNAP-25=synaptosomal-associated protein 25. VILIP-1=visinin-like protein 1. YKL-40=chitinase-3-like protein 1. p<0·05. *Aβ=amyloid β. †p<0·01. ‡p<0·001. §p<0·0001. Absolute mean differences and associated p values are shown in the appendix (pp 6–7).

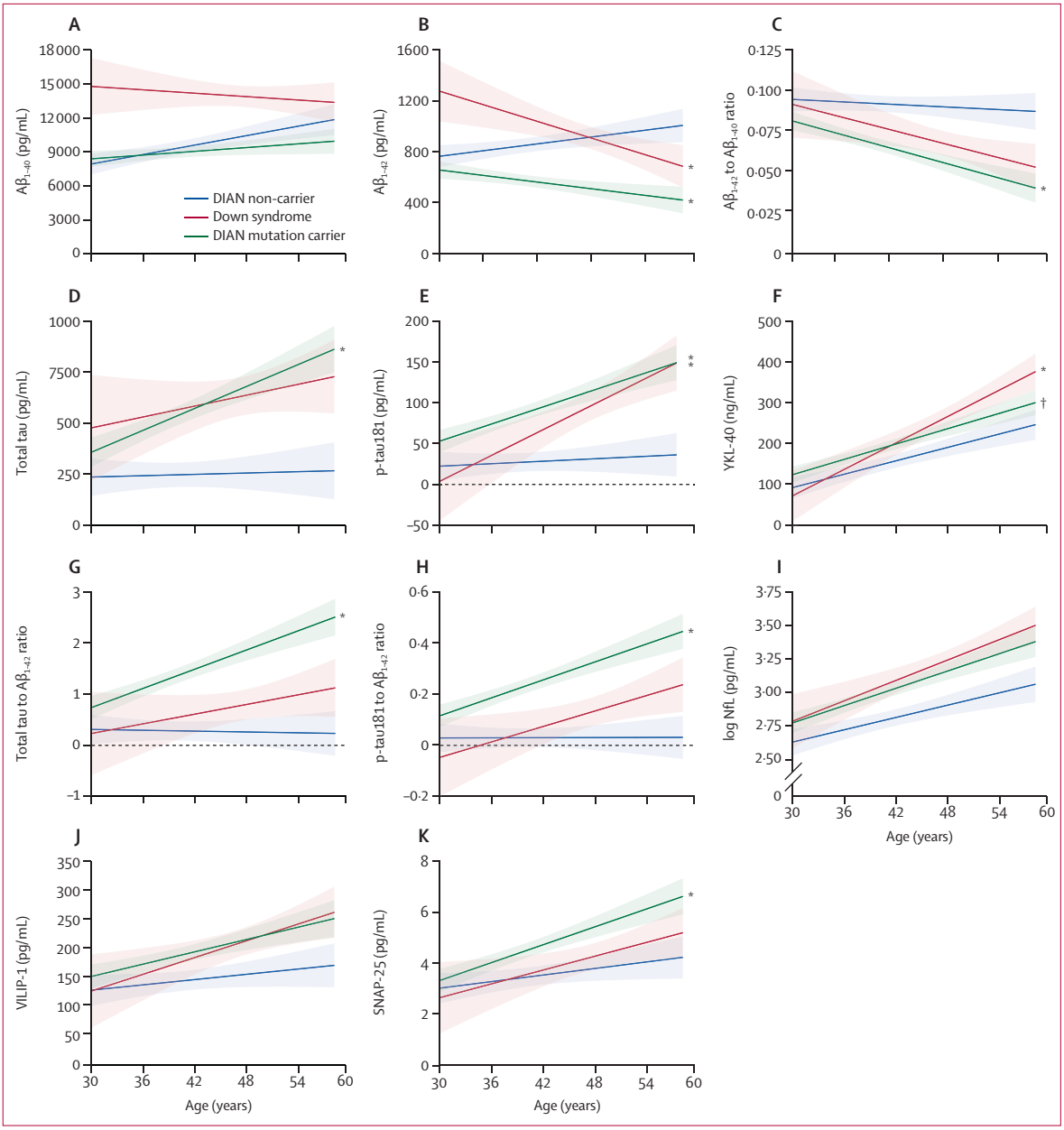

Figure 2: **CSF biomarkers of amyloid, tau, and phosphorylated tau-related processes, astrogliosis and neuroinflammation, and neuronal, synaptic, and axonal injury as a function of age**

Biomarkers included (A) $A\beta_{1-40}$, (B) $A\beta_{1-42}$, (C) $A\beta_{1-42}$ to $A\beta_{1-40}$ ratio, (D) total tau, (E) p-tau181, (F) YKL-40, (G) total tau to $A\beta_{1-42}$ ratio, (H) p-tau181 to $A\beta_{1-42}$ ratio, (I) log transformed NfL, (J) VILIP-1, and (K) SNAP-25. Coloured lines reflect the regression lines and 95% CIs based on the linear regression, with each biomarker as the outcome and *APOE* ε4 status, sex, and the group by age interaction as the covariates. Pair-wise comparisons of the change in biomarkers over age (slopes of the linear lines) controlling for *APOE* ε4 status and sex was performed based on the linear regressions, and p values for prespecified subgroup comparisons were adjusted by the Benjamini-Hochberg method.[14] Absolute mean differences in annualised slopes and associated p values are shown in the appendix (p 8). Aβ=amyloid β. APOE=apolipoprotein E. DIAN=Dominantly Inherited Alzheimer Network. NfL=neurofilament light chain. SNAP-25=synaptosomal-associated protein 25. VILIP-1=visinin-like protein 1. YKL-40=chitinase-3-like protein 1. *Significant (p<0·05) summary group differences in slope (Down syndrome or DIAN mutation carriers *vs* DIAN non-carrier). †Significant (p<0·05) summary group differences in slope (Down syndrome *vs* DIAN mutation carriers).

two genetic groups were observed. Individuals with Down syndrome showed overall higher concentrations of $A\beta_{1-40}$ than the other two groups across all ages (figure 2A). Despite similar slopes for $A\beta_{1-42}$ in the Down syndrome and DIAN mutation carrier groups versus the DIAN non-carrier group (both p<0·0001, although with a slightly greater association with age in Down syndrome [p<0·080]), like $A\beta_{1-40}$, overall amounts were higher in Down syndrome (figure 2B). Robust increases in p-tau181 with age were observed in both genetic groups versus DIAN non-carrier

controls (p=0·0030 for Down syndrome; p=0·0020 for DIAN mutation carriers; figure 2E), but the total tau slope in Down syndrome did not differ significantly from DIAN non-carriers despite overall higher concentrations (figure 2D). The tau to $A\beta_{1-42}$ slopes in Down syndrome were not different from the DIAN non-carrier group (total tau to $A\beta_{1-42}$, p=0·20; p-tau181 to $A\beta_{1-42}$ p=0·050; figure 2G, H). YKL-40 increased in all groups with age, but slopes were greater in Down syndrome versus DIAN non-carriers (p=0·020) and DIAN mutation carriers (p=0·030; figure 2F). NfL and VILIP-1 (figure 2I, J) increased in Down syndrome but, similar to those in the DIAN mutation carrier group, their slopes were not different from DIAN non-carrier controls. In contrast to those in the DIAN mutation carrier group, the pattern of SNAP-25 in Down syndrome did not differ from those in the DIAN non-carrier group (figure 2K). p values for pairwise comparisons are shown in the appendix (p 8). Down syndrome biomarker patterns in the karyotype groups are shown in the appendix (pp 10–11).

Discussion

Despite the differences in underlying causes for Alzheimer's disease development in the two at-risk genetic cohorts, adults with Down syndrome had CSF biomarker changes remarkably similar to carriers of autosomal dominant Alzheimer's disease mutations, and both consistent with expected accrual of Alzheimer's disease pathology with advancing age. Profiles included reductions in $A\beta_{1-42}$ to $A\beta_{1-40}$ ratio and increases in markers of phosphorylated tau-related processes; neuronal, axonal, or synaptic injury; and astrogliosis and neuroinflammation, with typically greater degrees of abnormality in the presence of dementia, as has been described in late-onset Alzheimer's disease[3,15] and autosomal dominant Alzheimer's disease.[5,16] CSF biomarkers have been reported in this cohort[10] and other Down syndrome cohorts,[2,7,17] but, to our knowledge, no direct comparisons have been made between individuals with Down syndrome and those with autosomal dominant Alzheimer's disease. Despite many similarities, we also observed some variations that could shed light on potential differences in Aβ metabolism, neuronal injury, and astrogliosis and neuroinflammation specifically in the setting of trisomy 21.

Elevations in CSF $A\beta_{1-40}$ and $A\beta_{1-42}$ in Down syndrome are likely to reflect the triplication of the *APP* gene, resulting in global increases in total APP, whereas increased $A\beta_{1-42}$ in autosomal dominant Alzheimer's disease is typically the result of altered secretase activity.[18,19] The exception would be rare mutations that result in the duplication of *APP*. Although their rarity in the current study allows for only preliminary conclusions to be drawn (29 patients who were DIAN mutation carriers were from families with *APP* mutations, only four of whom had *APP* duplications; appendix p 2), a direct comparison in a future larger cohort will be very informative. Comparability of the $A\beta_{1-42}$ to $A\beta_{1-40}$ ratio suggests similar timing of Aβ aggregation with respect to disease pathogenesis and progression in the two groups, a finding that could inform the timing of experimental interventions aimed at preventing dementia onset through reductions in amyloid.[3] Comparison of CSF biomarker profiles with amyloid PET is underway and will inform possible CSF diagnostic cutoff values in Down syndrome, which could be used to define amyloid status for clinical trials. Whether measures of plasma Aβ have utility as a more non-invasive biomarker of amyloid pathology in Down syndrome is also of great interest and remains to be established.[20]

In contrast to autosomal dominant Alzheimer's disease, amounts of total tau and NfL in Down syndrome were already elevated (compared with DIAN non-carriers) in the asymptomatic stage. Although the older age of the asymptomatic Down syndrome group might have contributed to this finding (both biomarkers are known to increase with age[21,22]), such a pattern might reflect or be influenced by the biological and neurodevelopmental differences associated with Down syndrome. Autopsy and antemortem imaging studies in Down syndrome have described reduced brain size, lower numbers and depth of cerebral sulci, enlarged ventricles, and hypoplasia of several brain regions in comparison to individuals without Down syndrome of similar ages.[23] With advancing age, additional volume reductions are observed in regions known to develop neurofibrillary tangle pathology.[24] Although the total tau and NfL patterns are consistent with such changes, direct comparisons of these fluid measures with structural and molecular imaging are required to fully understand their causes as well as whether there are relationships with the level of pre-existing intellectual disability. These topics are the focus of future studies. Studies from the past few years have reported elevations in plasma NfL in Down syndrome,[2,17,25] duplicating increases in CSF NfL. Despite potential age-related differences in patterns of total tau and NfL between the Down syndrome and DIAN mutation carrier groups, p-tau181 patterns are virtually identical, suggesting similar pathophysiological processes involved in tau hyperphosphorylation or aggregation, or both. The reason why tangle pathology is greater in both Down syndrome and autosomal dominant Alzheimer's disease than late-onset Alzheimer's disease[26,27] remains to be determined, but could be a consequence of elevated concentrations of brain $A\beta_{1-42}$ (since birth) in those with confirmed genetic mutations compared with those who develop late-onset Alzheimer's disease. Data from human neuroimaging and mouse models support a role of amyloid in fostering an environment favourable for the development of tau pathology.[28,29]

Inflammation is a key process in Down syndrome, probably because chromosome 21 contains several pro-inflammatory and anti-inflammatory genes. The brains of individuals with Down syndrome display an inflammatory

phenotype different from late-onset Alzheimer's disease,[30] and elevations in plasma inflammatory markers have been reported in Down syndrome.[31] In the current study, higher overall amounts of CSF YKL-40 in Down syndrome and more rapid elevations with age are consistent with a systemic and dysregulated inflammatory process, although age-related differences in the cohorts could also contribute to this observation. A study of a small cohort (n=12 Down syndrome, n=20 controls) reported no difference in YKL-40 between the age-matched groups (aged around 40 years), but increases with age in both groups, with significantly higher amounts in older (>40 years) adults with Down syndrome,[32] consistent with our results. Known correlations between CSF YKL-40 and total tau could also contribute.[3,5,10]

Given the ease, availability, and relative non-invasiveness of venipuncture compared with lumbar puncture in individuals with Down syndrome, it is probable that plasma or serum biomarkers—once fully validated—will be the most feasible modality for clinical trial screening and potential clinical care in this at-risk population. Development of reliable, highly sensitive assays for blood-based markers has enabled their evaluation in different Alzheimer's disease cohorts,[33–37] including Down syndrome.[2,17,31,38,39] Future comparisons of the plasma profiles among these groups will be informative as the field moves closer to bringing biomarkers to the clinic.

The major strength of the study is the comparison of CSF biomarkers in two of the most relevant cohorts of individuals with genetically determined forms of Alzheimer's disease; however, the study also has limitations. The number of participants with Down syndrome with available CSF samples (n=41), although larger than most previous studies, is still relatively small, limiting statistical power, especially regarding possible false-negatives. This cohort is also heterogeneous in terms of karyotype and racial characteristics, although no obvious differences were noted in subanalyses (appendix pp 9–11). Despite selecting DIAN participants based on the age range of the ABC-DS cohort, the mean ages turned out to be different. Not all samples had data for all biomarkers, and ABC-DS longitudinal data were not available. As in all Down syndrome studies, there is the inherent challenge of determining dementia in the presence of existing intellectual disability. Additionally, although the development of Alzheimer's disease pathology and risk of dementia increases with advancing age in both genetic groups, carriers of autosomal dominant Alzheimer's disease mutations typically develop dementia at different ages. The DIAN metric of estimated years to symptom onset permits assigning an individual a place along the disease trajectory without regard to chronological age, thus enabling stage-similar comparisons between individuals with different mutations.[4] No such metric yet exists for adults with Down syndrome given the variability in symptom onset and presentation, thus impeding the ability to make pathological stage-specific comparisons between the genetic groups. Longitudinal assessment of CSF biomarkers in ABC-DS participants as they progress from asymptomatic (cognitively stable) to symptomatic (dementia) stages will be very informative.

In conclusion, CSF biomarker patterns have many similarities in Down syndrome and autosomal dominant Alzheimer's disease, thus reflecting a common pathway in Alzheimer's disease pathophysiology independent of the underlying initial genetic cause. This finding supports their potential utility for the detection and tracking of Alzheimer's disease-related processes and suggests that treatments effective in one population could have utility in the other. Such knowledge might inform clinical trial design in these understudied groups at risk. However, the overall higher concentrations of Aβ and potential preclinical (presymptomatic) elevations in markers of neuronal injury (total tau) and astrogliosis and neuroinflammation (YKL-40) in Down syndrome highlight the inherent metabolic differences that should be considered when defining CSF cutoff values for identification of underlying Alzheimer's disease pathologies currently being used in late-onset Alzheimer's disease for trial enrolment and evaluation of target engagement or biomarker outcomes.

Contributors

AMF and RLH were responsible for the literature search. AMF, RLH, YL, CX, WEK, BLH, NS, ITL, ED, BMA, and BTC were responsible for study design. AMF, RLH, RJB, AG, ED, BTC, FL, HDR, NS, SKM, WS, JHL, WEK, BLH, RFA, JPC, GSD, NRGR, MJ, JL, RNM, CLM, HM, CJM, YN, JMR, SS, PS, MS, and ITL were responsible for data collection and verification. AMF, RLH, YL, AHB, and CX were responsible for data analysis. AMF, RLH, YL, AHB, CX, RJB, AG, BMA, NS, SKM, WS, JHL, WEK, BLH, ITL, and BTC were responsible for data interpretation. AMF, RLH, YL, and AHB were responsible for figures. AMF, RLH, and YL were responsible for manuscript writing. RLH, YL, AHB, CX, RJB, AG, BMA, ED, BTC, FL, HDR, NS, SKM, WS, JHL, WEK, BLH, RFA, JPC, GSD, NRGR, MJ, JL, RNM, CLM, HM, CJM, YN, JMR, SS, PS, MS, and ITL were responsible for manuscript critical review. AMF, RLH, YL, AHB, and CX had access to all the data in the study, and AMF had final responsibility for the decision to submit for publication.

Declaration of interests

AMF has received research funding from the National Institutes of Health/National Institute on Aging, Biogen, Centene, Fujirebio, and Roche Diagnostics. She is a member of the scientific advisory boards for Roche Diagnostics, Genentech, and AbbVie and also consults for Araclon/Grifols, DiademRes, DiamiR, and Otsuka Pharmaceuticals, outside the submitted work. RJB has equity ownership interest in C2N Diagnostics and receives royalty income based on technology (stable isotope labelling kinetics and blood plasma assay) licensed by Washington University to C2N Diagnostics. He receives income from C2N Diagnostics for serving on the scientific advisory board. Washington University, with RJB as co-inventor, has submitted the US non-provisional patent application "Cerebrospinal fluid (CSF) tau rate of phosphorylation measurement to define stages of Alzheimer's disease and monitor brain kinases/phosphatases activity." He has received honoraria from Janssen and Pfizer as a speaker, and from Merck and Pfizer as an advisory board member. He has been an invited speaker, advisory board member, and consultant for F Hoffman La Roche, an invited speaker and consultant for AC Immune and Janssen, and a consultant for Amgen and Eisai, outside the submitted work. AMG has consulted for Eisai, Biogen, Pfizer, AbbVie, Cognition Therapeutics, and GSK. She also served on the Scientific Advisory Board of Denali Therapeutics (2015–2018), outside the submitted work. BJH has received research funding from Roche Pharmaceuticals and Autism Speaks,

outside the submitted work. JPC has served on a medical advisory board for Otsuka Pharmaceuticals, outside the submitted work. GSD is supported by National Institutes of Health/National Institute on Aging (K23AG064029). He serves as a topic editor on dementia for DynaMed Plus (EBSCO Industries), a consultant for Parabon NanoLabs, is the clinical director for the Anti-NMDA Receptor Encephalitis Foundation (uncompensated), has provided record review and expert medical testimony on legal cases pertaining to management of Wernicke encephalopathy, and holds stocks (>$10 000) in ANI Pharmaceuticals (a generic pharmaceutical company), outside the submitted work. NRGR takes part in multicentre trials supported by AbbVie, Eli Lilly, and Biogen, outside the submitted work. JL reports speaker fees from Bayer Vital and Roche, consulting fees from Axon Neuroscience and Ionis Pharmaceuticals, author fees from Thieme medical publishers and W Kohlhammer GmbH medical publishers, non-financial support from Abbvie, and compensation for duty as part-time CMO from MODAG, outside the submitted work. CJM has been a member of advisory scientific board for Biogen, IONIS, Wave, and Roche and consulted for Eisai, outside the submitted work. RNM has received funding from the US Alzheimer's Foundation to undertake an intervention trial for the prevention of Alzheimer's disease. He is a member of the scientific advisory board for Eisai, outside the submitted work. SS reports consulting to Eisai, Novartis, Genentech, F Hoffmann-La Roche, Gemvax, Avid Radiopharmaceuticals, and Eli Lilly and Company, outside the submitted work. All other authors declare no competing interests.

Data sharing

De-identified, individual participant-level data that underlie the results reported in this Article (and associated data dictionaries) are available upon request to the respective studies (ABC-DS and DIAN) providing applications are approved by the separate steering committees. Requests must detail the study hypothesis and include a statistical analysis plan. Committee review will take into consideration the merit, feasibility, and scientific rigour of the proposed study. Study protocols and informed consent forms can also be requested. All applicants must sign a data use agreement that includes statements regarding sharing of data to a third party.

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For **ABC-DS** see https://pitt.co1.qualtrics.com/jfe/form/SV_cu0pNCZZlrdSxUN

For **DIAN** see https://dian.wustl.edu/our-research/for-investigators/DIAN observational-study-investigator-resources/

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