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RESEARCH ARTICLE

Alpha-synuclein co-pathology in Down syndrome-associated Alzheimer's disease

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Abstract

INTRODUCTION: Alpha-synuclein (αSyn) seed amplification assay (SAA) enables in vivo study of αSyn but remains underexplored in Down syndrome-associated Alzheimer's disease (DSAD).

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METHODS: We analyzed αSyn-SAA in cerebrospinal fluid (CSF) from 270 adults with Down syndrome, from the Down Alzheimer Barcelona Neuroimaging Initiative and from the AD21 cohort from the Department of Neurology at the University Hospital, Ludwig Maximilian University of Munich, Germany. Neuropathological examinations were conducted in 19 brain donors (five with *ante mortem* CSF). Participants were classified as asymptomatic or symptomatic (prodromal/dementia) Alzheimer's disease (AD). CSF Aβ1-42/1-40, CSF and plasma p-Tau181, and neurofilament light chain (NfL) levels were measured. Neuropathological evaluations assessed AD neuropathological changes and Lewy body pathology (LBP).

RESULTS: ASyn-SAA was positive in 9.2% of cases, independent of age or cognitive status. Symptomatic αSyn-positive cases exhibited higher plasma NfL levels than αSyn-negative cases (31 vs 21 pg/mL, $p = 0.027$). LBP was observed in 47% of necropsies. The individual with severe neocortical LBP was αSyn-SAA-positive.

DISCUSSION: These findings highlight LBP prevalence in DSAD but suggest current SAA may fail to detect limited αSyn deposition.

KEYWORDS
Alzheimer's disease, biomarker, Down syndrome, Lewy body pathology, neuropathology, seed amplification assay, α-synuclein

Highlights

- αSyn-SAA positivity in DSAD is 9.2%, similar to ADAD but lower than sporadic AD.
- Misfolded αSyn was detectable from early ages in individuals with DS.
- Positivity rates did not vary with age or clinical status in DS.
- Plasma NfL levels are higher in symptomatic αSyn-SAA positive versus negative cases.
- CSF αSyn seeding activity was associated with high neocortical LBP at necropsy.

1 | INTRODUCTION

Down syndrome (DS) is a genetic form of Alzheimer's disease (AD), with virtually all individuals developing AD pathology in the fourth decade of life due to the triplication of chromosome 21, which includes the gene encoding amyloid precursor protein *(APP)*.[1–3] This overexpression of *APP* leads to the accumulation of AD neuropathological changes, including extracellular amyloid beta (Aβ) plaques, intracellular aggregates of hyperphosphorylated tau, and widespread synaptic and neuronal degeneration.[1] In addition to these core pathologies, DS-associated AD (DSAD) often includes aggregates of misfolded α-synuclein (αSyn) in the form of Lewy bodies (LB) and Lewy neurites, adding complexity to the neuropathological landscape.[4,5]

Post mortem studies have identified Lewy body pathology (LBP) in 27% to 85% of brains from autosomal dominant AD (ADAD) cases and 31% to 54% of sporadic AD (sAD) cases, with significant variability across cohorts.[6] Notably, αSyn immunoreactivity in AD is often localized in the amygdala, resulting in an amygdala-predominant LBP (Amg-LBP) pattern that contrasts with the Braak staging seen in Parkinson's disease and dementia with Lewy bodies (DLB).[7] In addition, other patterns of distribution of LBP have been described, including widespread cortical LBP. It has been speculated that LBP in cases of genetically determined AD may represent a secondary phenomenon driven by AD pathology that promotes αSyn aggregation in susceptible regions.[6]

Knowledge of LBP in DSAD derives from *post mortem* studies,[4,5] leaving critical gaps in understanding the timing and clinical implications of αSyn accumulation. The αSyn seed amplification assay (αSyn-SAA) has emerged as a highly precise tool for detecting pathological αSyn in vivo. Previous studies reported αSyn-SAA positivity in 6% to 11% of ADAD cases[8,9] and 21% to 45% of sAD cases.[10,11] However, the presence of LBP detected by αSyn-SAA in DSAD remains unexplored.

This study investigates the presence of LBP in DSAD using αSyn-SAA and evaluates its relationship with fluid biomarkers and neuropathological findings. By integrating in vivo and *post mortem* data, we aim to elucidate the role of αSyn pathology in DSAD.

2 | METHODS

2.1 | Study participants

This study had two components: clinical and neuropathological. The first involved adults aged 18 years or older from two different cohorts, the population-based Down Alzheimer Barcelona Neuroimaging Initiative (DABNI)[12] from the Alzheimer-Down Unit at the Catalan Down Syndrome Foundation and the Hospital of Sant Pau (n = 258) and the AD21 cohort[13] from the Department of Neurology at the Ludwig Maximilian University (LMU) Munich Hospital (n = 12). Both units run population-based health programs in individuals with DS. These plans are focused on neurological conditions, especially AD. Patients generally undergo structured neurological and neuropsychological evaluations on a semi-annual or annual basis, conducted by experienced clinicians. Only those with available cerebrospinal fluid (CSF) samples were included, representing both males and females from both cohorts. We included a control group of sex- and age-matched individuals (n = 20) with no history of major neurological or psychiatric disorders, all of whom demonstrated normal cognitive function and neurological status upon examination. All participants were evaluated by board-certified neurologists, who systematically gathered clinical and demographic data. Eligible control participants were enrolled between 2020 and 2023 at the Department of Neurology, LMU Munich Hospital. All subjects participated in a structured research protocol with periodic clinical follow-ups, and each had at least one reassessment after their baseline visit.

The second component focused on *post mortem* analyses, involving 19 deceased brain donors with DS whose neuropathological data were sourced from the Neurological Tissue Bank of the Biobank, Hospital Clínic-FRCB/IDIBAPS, Barcelona, Spain. Some donors (n = 5) had previously undergone lumbar puncture, and *ante mortem* CSF samples were available for analysis .

The study was conducted in accordance with the principles of the Declaration of Helsinki and received approvals from the Sant Pau Ethics Committee and the local ethics committee of the LMU medical faculty. Written informed consent was obtained from all participants or their legally authorized representatives. Confidentiality was safeguarded following current legal frameworks.

2.2 | Procedures

Participants with DS underwent a comprehensive evaluation to determine their clinical and cognitive status. This evaluation included a semi-structured interview with caregivers, a detailed neurological examination, and a neuropsychological assessment. As part of the cognitive evaluation, patients who were cognitively capable of undergoing neuropsychological assessment were administered the Cambridge Cognitive Examination for Older Adults with Down Syndrome (CAMCOG-DS), the German version[14] in Munich, and the Spanish version[15] in Barcelona.

RESEARCH IN CONTEXT

1. **Systematic review**: We reviewed the literature in PubMed, Scopus, and Web of Science on αSyn SAA for detecting LBP. While studies address Parkinson's disease, dementia with Lewy bodies, and AD, including autosomal dominant forms of AD, no research has explored DSAD.
2. **Interpretation**: Our findings indicate that misfolded αSyn positivity is detectable early in DSAD and persists consistently across AD stages. This contrasts with sporadic AD, where LBP prevalence increases with age and disease progression. SAA activity was linked to substantial neocortical LBP, being sensitive to detect pathology in these regions.
3. **Future directions**: Future studies should expand longitudinal analyses of LBP in DSAD, examine sex-based differences in prevalence, and analyze misfolded αSyn-SAA kinetics in DSAD. Investigating the clinical impact of αSyn aggregates and its correlations with multimodal biomarkers is also crucial.

The diagnostic classification of individuals was determined by consensus between two experienced clinicians: a neurologist and a neuropsychologist in Barcelona and two neurologists in Munich. Each specialist independently assessed the participants while remaining blinded to the other's evaluations and to the participants' biomarker status. Participants were subsequently classified into the following categories: asymptomatic, defined as having no evident acquired cognitive symptoms; prodromal AD, characterized by cognitive decline with preserved baseline functionality; and dementia AD, identified by cognitive impairment resulting in impaired functionality.[16] Intellectual disability (ID) level was categorized as mild, moderate, severe, or profound according to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition. This classification relied on caregivers' reports of the individual's highest level of functioning achieved. Additionally, for asymptomatic participants, scores from the Kaufman Brief Intelligence Test Spanish version[17] were considered in DABNI, and in the AD21 cohort the colored progressive matrices (CPM)[18] was initially used, but, due to outdated norms, it was replaced with the more recent Raven's Progressive Matrices 2 (Raven's 2).[19] Apolipoprotein E (*APOE*) ε4 status was determined by Sanger sequencing of polymorphisms rs429358 and rs7412 in exon 4 only for DABNI participants.[16,20]

2.3 | Cerebrospinal fluid and plasma analyses

CSF and blood samples were collected concurrently. Due to platform discrepancies, only CSF and plasma biomarkers from the DABNI cohort were included. The detailed protocol for sample collection was previously reported.[21] The concentration of CSF biomarkers, including Aβ1-42, Aβ1-40, and tau phosphorylated at threonine 181 (p-Tau181), were measured in the Lumipulse automated platform (Fujirebio—Europe)

using established methods; subsequently, Aβ1-42/Aβ1-40 ratio was calculated. Neurofilament light chain (NfL) in CSF concentrations was measured with a commercial ELISA (UmanDiagnostics, Umeå, Sweden), following the manufacturer's recommendations. Plasma concentrations of p-Tau181 and NfL were measured using a single-molecule array (Simoa; Quanterix) according to the manufacturer's instructions.

2.4 | α-synuclein seed amplification assay

CSF αSyn-SAA, utilizing real-time quaking-induced conversion (RT-QuIC) with recombinant WT human αSyn, was performed as previously described[22] in an academic setting. Positive and negative control samples were included in each experiment to ensure consistent comparison of fluorescent signal responses across plates. Positive controls consisted of CSF samples from two individuals with clinical and neuroradiological normal pressure hydrocephalus, demonstrating high αSyn seeding activity (4/4 positive wells in at least 10 consecutive runs). Negative controls consisted of CSF from individuals without clinical or neuropathological evidence of neurodegenerative disease, consistently exhibiting negative RT-QuIC responses (0/4 positive wells). To mitigate batch-to-batch and plate-to-plate variability, relative fluorescent units were normalized to the median maximum intensity (Imax) of the four positive control replicates within each plate, expressed as a percentage. A threshold of 15% of the average maximum fluorescent intensity of the positive control was established, with a cutoff time of 144 h. All samples were measured in quadruplicates. A CSF sample was classified as positive if at least two of four replicates crossed this threshold. Samples with no positive replicates were considered negative. If one of four replicates reached the threshold, the sample was repeated once. If the repeated measurement also yielded only one positive well, the sample was classified as inconclusive to avoid potential false positives. All αSyn-SAA analyses were conducted at LMU Munich, Department of Neurology, Germany, by personnel blinded to participant clinical status.

2.5 | Neuropathological examination

Neuropathological examinations were conducted following standardized protocols[23] of brain donors of the Neurological Tissue Bank, Biobank-Hospital Clínic-FRCB/IDIBAPS, Barcelona, Spain. Immunohistochemistry utilized the following primary antibodies: anti-βA4 (6F/3D, Dako), anti-tau (AT8, Thermo Fisher Scientific), and anti-αSyn (5G4, Analytik Jena). LBP was assessed semi-quantitatively (0 = absent; 0.5 = rare; 1 = mild; 2 = moderate; 3 = severe) in the following regions: olfactory bulb, amygdala, dorsal motor nucleus of the vagus, locus coeruleus, substantia nigra, subiculum/CA1 region of the hippocampus, anterior cingulate gyrus, and frontal and parietal cortices. Disease evaluations were performed according to National Institute on Aging-Alzheimer's Association (NIA-AA) consensus criteria for AD,[24] McKeith criteria[25] following BrainNet Europe Consortium guidelines,[26] and the 2021 neuropathological consensus criteria for LBP.[27] LBP density assessment was performed using as reference the BrainNet Europe Consortium guidelines[26] for the mild, moderate, and severe categories. The rare category was stated when isolated immunoreactivity was observed in the whole sample, and a negative result could not be given but was considered insufficient to categorize as mild.

2.6 | Statistical analysis

Baseline sociodemographic and biomarker characteristics were summarized as follows: categorical variables (e.g., sex, ID level, and *APOE*-ε4 status) were reported as absolute frequencies and percentages. Unless otherwise specified, continuous variables, such as age and CSF and plasma biomarker levels, were summarized using medians and interquartile ranges. Differences between αSyn-SAA-positive and -negative results were analyzed for the entire cohort and stratified by the presence of cognitive symptoms. Prodromal and dementia groups were combined for analysis as one single symptomatic group, as no αSyn-SAA-positive results were observed in the prodromal AD stage. Detailed results classified by clinical stage are provided in the supplementary material. Normality was assessed using the Shapiro-Wilk test. As data were not normally distributed, the Mann–Whitney U test was used for continuous variables. For categorical variables, the chi-squared test or Fisher's exact test was applied as appropriate. Statistical significance was set at $\alpha = 0.05$, and analyses were conducted using R (version 4.2.2).

3 | RESULTS

3.1 | CSF αSyn-SAA results and stratification

We included 270 individuals with DS spanning the entire AD continuum, comprising 106 asymptomatic individuals and 140 symptomatic (51 in the prodromal stage, and 89 in the dementia stage). Twenty-three cases had an uncertain cognitive impairment etiology due to confounding conditions (e.g., psychiatric or medical comorbidities). Positive CSF αSyn-SAA results were identified in 23 samples, while nine cases yielded inconclusive results. All controls (n = 20, mean age 43.5 ± 19.4 years) without evidence of neurodegenerative disease yielded negative results (Table S1). Subjects with cognitive impairment of uncertain etiology (21 αSyn-SAA-negative, one αSyn-SAA-inconclusive, and one αSyn-SAA-positive) were excluded from further analyses, along with all other inconclusive results. To clarify, the single positive case with uncertain etiology was excluded from the final analysis. Refer to Figure S1 for the flowchart illustrating sample composition.

3.2 | Demographic and biomarker profiles

Table 1 summarizes the demographic characteristics and fluid biomarker data of participants, categorized by the presence of AD

TABLE 1 Demographic characteristics and fluid biomarkers stratified by the presence of Alzheimer's disease symptoms and αSyn-SAA status.

	Asymptomatic			Symptomatic			Total		
Characteristics	αSyn-SAA negative *n* = 92	αSyn-SAA positive *n* = 10	*P*	αSyn-SAA negative *n* = 125	αSyn-SAA positive *n* = 12	*P*	αSyn-SAA negative *n* = 217	αSyn-SAA positive *n* = 22	*p*
Age (years)	38.0 [30.8, 45.0]	37.5 [32.0, 48.3]	0.8	52.0 [48.0, 56.0]	49.5 [45.0, 55.3]	0.3	48.0 [41.0, 52.0]	47.0 [39.3, 51.8]	0.6
Sex (female)	37 (40%)	5 (50%)	0.7	49 (39%)	8 (67%)	0.075	86 (40%)	13 (59%)	0.077
Intellectual disability			0.4			>0.9			0.7
Mild	35 (38%)	2 (20%)		19 (16%)	2 (17%)		54 (25%)	4 (18%)	
Moderate	46 (51%)	7 (70%)		75 (62%)	8 (67%)		121 (57%)	15 (68%)	
Severe/profound	10 (11%)	1 (10%)		27 (22%)	2 (17%)		37 (17%)	3 (14%)	
***APOE ε4* carrier**	13 (14%)	2 (20%)	0.6	29 (25%)	1 (9.1%)	0.5	42 (20%)	3 (14%)	0.8
CSF Aß1-42/1-40 ratio	0.079 [0.065, 0.095]	0.080 [0.071, 0.086]	>0.9	0.044 [0.039, 0.051]	0.044 [0.041, 0.048]	0.8	0.053 [0.041, 0.077]	0.053 [0.044, 0.081]	0.5
CSF pTau181 (pg/mL)	29.4 [18.2, 43.4]	22.1 [17.9, 49.9]	0.7	135.9 [79.0, 198.0]	120.3 [77.4, 164.4]	0.6	72.5 [31.1, 155.5]	62.6 [22.2, 120.3]	0.5
CSF NfL (pg/mL)	374.2 [219.0, 532.2]	328.6 [246.0, 480.3]	0.7	1,050.8 [746.1, 1,504.8]	1,122.0 [752.1, 1,886.8]	0.5	721.4 [428.0, 1,221.6]	593.3 [328.6, 1,094.5]	0.5
Plasma pTau181 (pg/mL)	11.0 [7.9, 15.4]	10.8 [8.2, 12.3]	0.8	20.8 [13.8, 30.7]	22.4 [20.2, 30.9]	0.4	15.4 [9.8, 23.5]	14.7 [11.4, 22.4]	>0.9
Plasma NfL (pg/mL)	8.4 [5.3, 12.6]	9.0 [5.1, 12.4]	0.9	21.1 [15.4, 26.3]	31.0 [23.1, 36.2]	0.027	16.1 [9.0, 23.2]	14.2 [9.0, 27.6]	0.9

Notes: Data are *n* (%) or median [IQR].

Abbreviations: αSyn-SAA, α-synuclein seed amplification assay; Aβ, amyloid beta; APOE, apolipoprotein E; CSF, cerebrospinal fluid; NfL, neurofilament light chain; pTau, phosphorylated tau.

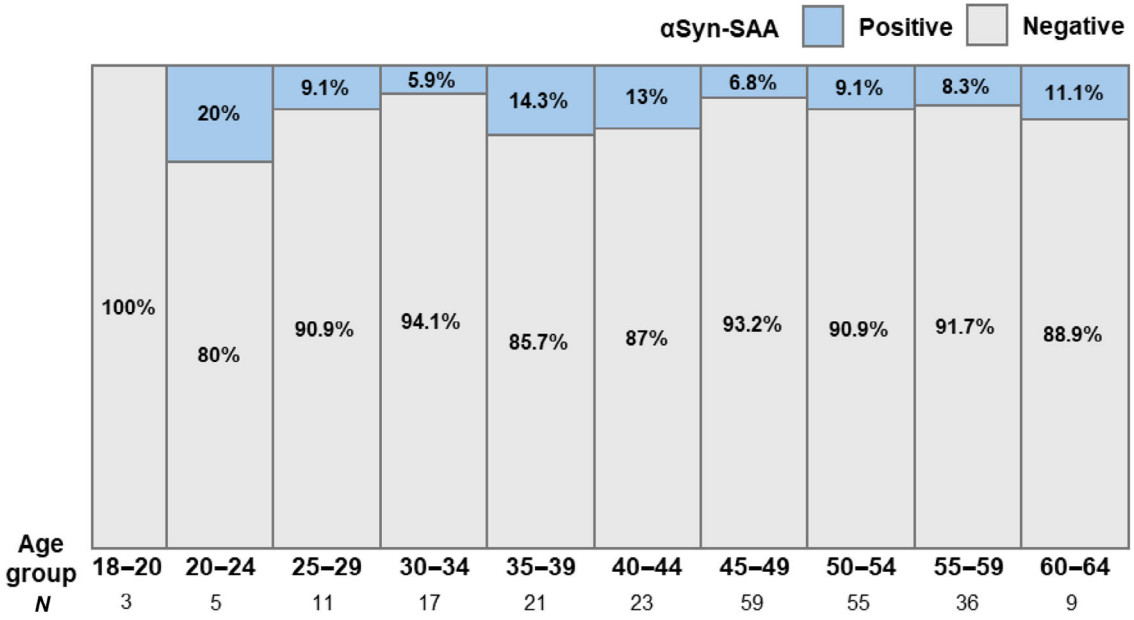

FIGURE 1 Distribution of αSyn-SAA results across age ranges. Point prevalence of positive and negative αSyn-SAA results in individuals with Down syndrome. αSyn-SAA, α-synuclein seed amplification assay.

symptoms, while Table S2 presents these data according to AD clinical stage. Tables S3 and S4 present clinical and demographic data for each individual with DS with a positive and inconclusive result. Positive αSyn-SAA results were similar in the asymptomatic and symptomatic groups (9.8% vs 8.8%; $p = 0.8$). The proportion of positive results remained stable across the age spectrum (Figure 1). Males exhibited a trend for lower positivity rates than females (6.4% vs 12.7%; $p = 0.077$). Plasma NfL levels were significantly elevated in SAA-positive versus SAA-negative individuals with cognitive impairment (31 vs 21 pg/mL; $p = 0.027$). However, no other significant differences in CSF biomarker concentrations were found based on αSyn-SAA status in the whole sample or after stratification by the presence of symptoms (Figure 2), or by clinical AD stage (Figure S2).

3.3 | Neuropathological findings

Table 2 presents the neuropathological data. The mean age at death was 56.2 years, with 52.6% of donors being female. Of these, five (26.3%) had not exhibited dementia symptoms during life. Amyloid deposition consistent with Thal stage 5 was observed in all but two individuals (89.5%), and tau pathology corresponding to Braak stages V and VI was present in all but four cases (78.9%). Frequent neuritic plaques according to Consortium to Establish a Registry for Alzheimer's Disease criteria were observed in all but one case (94.7%). LBP was identified in nine individuals (47.4%) (Figure 3). Based on the McKeith criteria,[25,26] two cases were classified as olfactory bulb-predominant, five as amygdala-predominant, and two as neocortical. When reclassified using the most recent neuropathological consensus criteria for LBP,[27] three cases shifted from the amygdala-predominant to the neocortical category due to the presence of minimal or sparse LBP in frontal or parietal cortices.

3.4 | *Ante mortem* CSF and neuropathological correlation

Ante mortem CSF samples were available for five brain donors, with a mean interval of 4.7 years between CSF collection and death. The sole individual with abundant neocortical LBP presented a positive αSyn-SAA result. Among the four individuals with negative αSyn-SAA results, two showed no LBP in any region. The remaining two were classified as amygdala-predominant: One exhibited rare LBP density in the amygdala, while the other presented with a severe burden in the olfactory bulb, mild burden in the amygdala and locus coeruleus, and rare density in other analyzed regions, except for the frontal cortex, where LBP was absent.

4 | DISCUSSION

This is the first report of αSyn-SAA in CSF in individuals with DS and its correlation to neuropathological examination. Neuropathological findings confirmed AD pathology in all DS brain donors, with 47% showing LBP and 10.5% having neocortical LB deposits. The only individual with severe LBP in neocortex, tested positive for αSyn-SAA in *ante mortem* CSF.

Neuropathological examinations confirmed AD pathology in all DS brain donors, consistent with cortical amyloid deposition starting from adolescence in the form of diffuse amyloid plaques until full-blown AD pathology in all individuals by the age of 40.[1] The prevalence of LBP aligned with previous reports in DS.[4] Variability

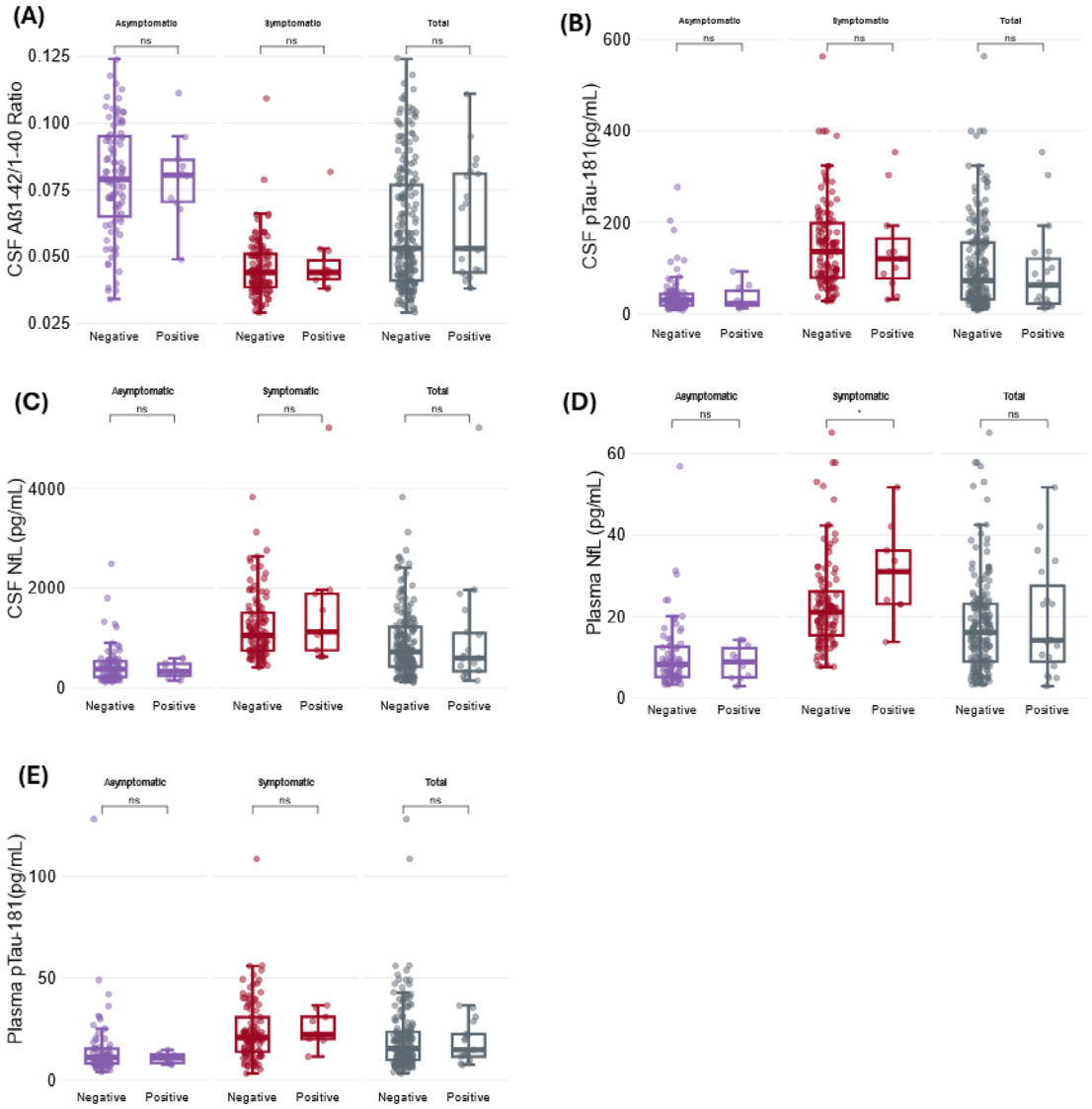

FIGURE 2 Fluid biomarker levels stratified by presence of Alzheimer's disease symptoms and aSyn-SAA status. Panels depict Aβ1-42/1-40 ratio levels in CSF (A), CSF concentrations of pTau-181 (B), and NfL (C) and plasma concentrations of NfL (D) and pTau-181 (E) based on the absence/presence of cognitive symptoms and aSyn-SAA outcomes. For each boxplot, the box represents the interquartile range, the band the median value, and the dots individual values. Statistical comparisons between groups were performed using the Wilcoxon rank-sum test. Significance is set at $p < 0.05$. *, $p < 0.05$. αSyn-SAA, α-synuclein seed amplification assay; Aβ, amyloid beta; CSF, cerebrospinal fluid; NfL, neurofilament light chain; ns, no significance; pTau, phosphorylated tau.

in LBP staging was observed depending on the classification system applied. Among the five individuals with any neocortical LB deposits, only two retained this classification under the McKeith criteria.[25,26] The remaining three, with rare or mild LB pathology, were classified as amygdala-predominant. The amygdala and olfactory bulb were the most frequently affected regions, which may explain the lower frequencies reported in other groups that excluded these regions.[5] Comparisons with other genetically determined forms of AD are challenging due to the wide variability in LBP prevalence (27% to 85%), likely influenced by the limited sample sizes in studies. Still, within the context of AD, the amygdala consistently emerged as the predominantly affected region by LB in both genetic forms of the disease.[6]

Among the five donors with *ante mortem* CSF samples available, no false positives were observed. Only one donor – exhibiting abundant LBP across all examined regions – showed positive seeding activity. Another individual, with rare to minimal αSyn pathology across most regions (except for severe involvement in the olfactory bulb and absence in the frontal cortex) tested negative, likely due to the low density of overall pathology, or possibly because widespread involvement occurred after CSF acquisition. These findings are consistent with reports from other authors indicating that αSyn-SAA sensitivity is reduced primarily in cases where αSyn pathology is restricted to focal regions outside the neocortex, such as the olfactory bulb, lower brainstem, or amygdala-predominant stages. In contrast, individuals at the limbic/transitional stage typically exhibit high αSyn-SAA sensitivity (95% to 100%).[28,29]

TABLE 2 Neuropathological characteristics of the brain donors with Down syndrome.

Case	Sex	Age death (y)	Brain weight (g)	Dementia	ABC score	Thal stage	Braak stage	Neuritic plaques	McKeith criteria	OB	Amy	DMNV	LC	SN	CA1/ subic	ACG	FC	PC	aSyn-SAA	CSF to death (y)
1	F	26	940	No	A3B0C1	5	0	Sparse	Negative	0	0	0	0	0	0	0	0	0	–	–
2	F	36	1170	No	A2B1C3	3	I	Frequent	Negative	0	0	0	0	0	0	0	0	0	–	–
3	F	43	975	No	A3B3C3	4	V	Frequent	Negative	0	0	0	0	0	0	0	0	0	Negative	6.66
4	M	44	1165	No	A3B1C3	5	I	Frequent	Negative	0	0	0	0	0	0	0	0	0	–	–
5	M	52	960	Yes	A3B3C3	5	VI	Frequent	Amy-pred	3	1	0.5	1	0.5	0.5	0.5	0	0.5	Negative	5.46
6	M	55	1055	Yes	A3B3C3	5	VI	Frequent	Negative	0	0	0	0	0	0	0	0	0	Negative	1.20
7	F	56	980	Yes	A3B3C3	5	VI	Frequent	Amy-pred	0	0.5	0	0	0	0	0	0	0	Negative	6.17
8	M	59	825	Yes	A3B3C3	5	VI	Frequent	Negative	0	0	0	0	0	0	0	0	0	–	–
9	F	59	940	Yes	A3B3C3	5	VI	Frequent	Amy-pred	1	1	0	0	0	0	0	0	0	–	–
10	F	60	845	Yes	A3B3C3	5	VI	Frequent	OB	1	0	0	0	0	0	0	0	0	–	–
11	M	61	980	Yes	A3B3C3	5	VI	Frequent	Neocortical	3	3	1	0.5	2	3	3	1	2	–	–
12	M	62	805	Yes	A3B3C3	5	VI	Frequent	Amy-pred	3	3	1	1	2	1	3	1	0.5	–	–
13	F	62	785	Yes	A3B3C3	5	VI	Frequent	Amy-pred	3	3	0.5	0.5	1	1	2	0	1	–	–
14	M	63	960	Yes	A3B3C3	5	VI	Frequent	OB	1	0	0	0	0	0	0	0	0	–	–
15	F	64	915	Yes	A3B3C3	5	VI	Frequent	Negative	0	0	0	0	0	0	0	0	0	–	–
16	F	64	850	Yes	A3B3C3	5	V	Frequent	Neocortical	3	3	3	3	3	3	3	3	3	Positive	3.89
17	M	64	975	Yes	A3B3C3	5	VI	Frequent	Negative	0	0	0	0	0	0	0	0	0	–	–
18	F	67	970	Yes	A3B3C3	5	V	Frequent	Negative	0	0	0	0	0	0	0	0	0	–	–
19	M	71	1025	No	A3B2C3	5	IV	Frequent	Negative	0	0	0	0	0	0	0	0	0	–	–

Note: The table summarizes demographic data, brain weight, and clinical dementia status at the time of death. AD pathology is classified using the ABC score, incorporating Thal amyloid phases, Braak stages, and the frequency of neuritic plaques. The semiquantitative burden of LBP is assessed based on LB density as follows: 0 = absent; 0.5 = rare; 1 = mild; 2 = moderate; 3 = severe. Results of CSF aSyn-SAA and the interval between LP and death are provided for available cases.

Abbreviations: αSyn-SAA, α-synuclein seed amplification assay; ACG, anterior cingulate gyrus; AD, Alzheimer's disease; Amy, amygdala; Amy-pred, amygdala-predominant; CA1/subic, CA1/subiculum; CSF, cerebrospinal fluid; DMNV, dorsal motor nucleus of the vagus; F, female; FC, frontal cortex; g, grams; LB, Lewy body; LBP, Lewy body pathology; LC, locus coeruleus; M, male; OB, olfactory bulb; PC, parietal cortex; SN, substantia nigra; subic, subiculum; y, years.

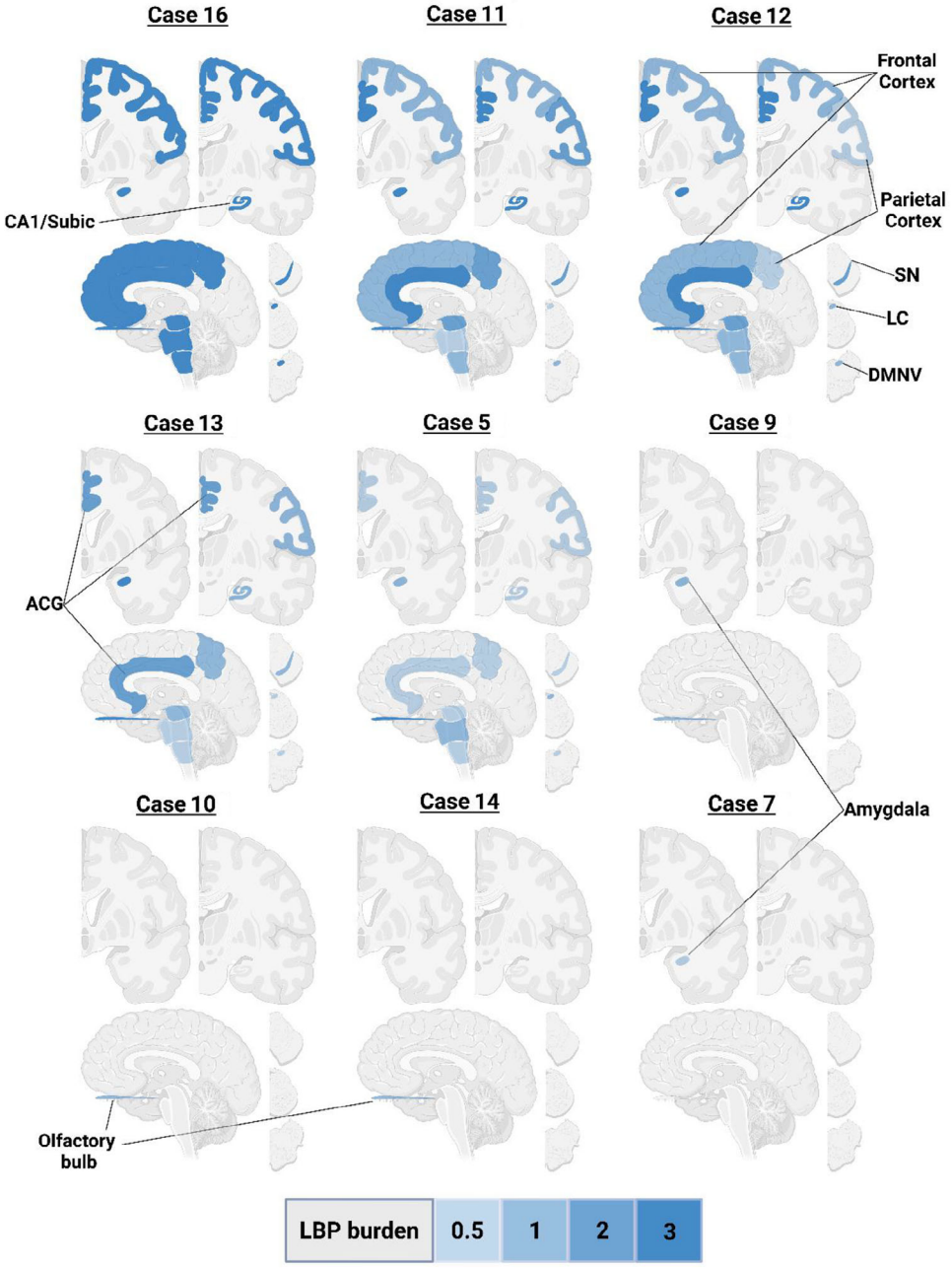

FIGURE 3 Regional distribution of LBP in neuropathological examination. Illustration of the regional distribution and density of LBP in cases with confirmed LB presence on neuropathological evaluation. The color scale on the brain maps represents semiquantitative scores for each anatomical region analyzed (0 = absent; 0.5 = rare; 1 = mild; 2 = moderate; 3 = severe). This model is an illustrative representation of areas based on the anatomical regions but not the specific regions analyzed in neuropathological examination. Created in https://BioRender.com. ACG, anterior cingulate gyrus; DMNV, dorsal motor nucleus of the vagus; LB, Lewy bodies; LBP, Lewy body pathology; LC, locus coeruleus; SN, substantia nigra; Subic, subiculum.

The overall positivity for αSyn seeding activity was 9.2%. The observed αSyn-SAA frequencies align with those previously reported in genetically determined AD cohorts (6% to 11%)[8,9] but remain markedly lower than the prevalence observed in sAD (21% to 45%).[10,11] This difference could be attributable to the predominant topographic deposition of LBP in the amygdala in these genetic populations. The typical Braak LBP distribution is more frequent in advanced age and more frequently found in late-onset sAD.[6,30–32] Of note, amygdala-predominant LBP, which seems to have lower seeding activity, is also more commonly observed in early-onset AD.[30]

The association between age and positive αSyn-SAA in sAD remains unclear. In our study, we observed a consistent prevalence of approximately 10% across the age spectrum. These findings are intriguing, as prior studies reported no positive αSyn-SAA results in 20 euploid controls, consistent with the high specificity observed in other SAA studies.[33] Additionally, we did not identify any false positives in indi-

viduals who underwent neuropathological examination. A possible explanation for αSyn aggregation in younger individuals with DSAD is the genetically driven nature of the disease, with AD-related pathology likely beginning decades before detection by conventional biomarkers. Although our analysis did not reveal a relationship between CSF amyloid burden and αSyn positivity – consistent with findings from other sAD cohorts[7–9] – recent studies demonstrated a significant association between αSyn positivity and higher Aβ burden.[10,11] In this line, as previously reported, Aβ deposits may begin accumulating as diffuse plaques as early as the third decade of life,[1] and elevated p-Tau levels in extracellular vesicles have been observed in asymptomatic DS individuals long before detectable increases in CSF or plasma.[12,34,35] These observations suggest that αSyn aggregation might begin in a preclinical phase, even in individuals without changes in traditional biomarkers, potentially reflecting an underlying pathological environment conducive to αSyn seeding activity. However, in our study, neocortical αSyn deposits were found only in individuals aged 61 and 64, consistent with prior reports of LBP in individuals over 50 years old.[5] This suggests that, although αSyn aggregation may occur at earlier stages in DSAD, the progression and distribution of LBP could still follow an age-dependent trajectory similar to that observed in ADAD.[9] Nevertheless, further studies are needed to determine whether these patterns reflect distinct pathogenic processes or a continuum within DS-related neurodegeneration.

Interestingly, the prevalence of positive SAA results was not related to AD symptoms. This is in contrast with findings in ADAD studies, where seeding activity was exclusively present in the CSF of symptomatic cases.[8,9] The observed lack of concordance may be partly attributable to the small sample sizes in prior studies, which included only 18 and 27 patients in the two investigations analyzing αSyn-SAA in ADAD. In the first, the only SAA-positive individual was a young woman (26 years old) who already exhibited cognitive symptoms.[8] Other genetically determined AD studies did not provide age data, although all SAA-positive cases were symptomatic.[9] Few authors have examined the prevalence of αSyn-SAA positivity across the clinical stages of AD. Two studies reported an increasing prevalence across cognitive stages: unimpaired, mild cognitive impairment (MCI), and dementia. Notably, MCI represented the largest group in both studies. Furthermore, biomarkers of amyloid pathology were positive in only approximately 50% of cases, suggesting the potential inclusion of individuals with etiologies other than AD.[36,37] In contrast, Pilotto et al. reported comparable SAA positivity rates between individuals with MCI and those with dementia due to AD, both significantly higher than those observed in non-AD controls.[11] Meanwhile, Bellomo et al. found similar prevalence rates in preclinical and MCI-AD stages, with higher rates observed at the dementia stage.[38] In our cohort, none of the prodromal AD cases exhibited positive SAA results. This discrepancy could stem from the limited group size but might also reflect the clinical impact of LBP, as prodromal AD patients with DS with significant LBP may rapidly progress to fulfill criteria for dementia (of note, dementia is usually diagnosed only 2 to 3 years later than prodromal AD).[12]

CSF and plasma biomarkers of AD pathology did not show significant differences in relation to positive seeding activity. To our knowledge, no other studies have examined the association of αSyn-SAA and CSF biomarker levels in genetically determined AD individuals, although similar findings have been observed in other cohorts.[10,11,38] However, as commented above, two recent studies reported that αSyn-positive participants exhibited a higher Aβ burden.[36,37] Additionally, plasma NfL levels (but not CSF) were higher in SAA-positive symptomatic individuals, potentially reflecting the existence of a greater neurodegeneration in the presence of αSyn. The discrepancy between plasma and CSF NfL levels remains unclear, although plasma NfL has been shown to exhibit earlier changes along the DSAD continuum.[12] Additionally, differences in assay sensitivity between CSF and plasma may partly account for this observation.

Despite the significance of these findings and the large sample size of the study, replication in other cohorts is necessary. Future studies should aim to include more diverse populations, as the demographic composition of our cohorts, predominantly White, reflects the characteristics of the populations from which participants were recruited. One controversial point is the observed trend toward a higher proportion of positive seeding activity in women, which contrasts with findings from other cohorts.[37] While not statistically significant, this trend warrants further investigation. At this stage, we can only hypothesize that biological or hormonal factors might contribute to these differences, particularly in advanced stages of the disease. Beyond this, future analyses should focus on longitudinal evaluations of αSyn seeding activity to provide more detailed insights into LBP progression in DSAD and clarify the reasons behind the relatively high prevalence of inconclusive results. Our data suggest a complex interplay between age, AD pathology, and αSyn aggregation and do not allow for definitive conclusions regarding an age-dependent trajectory of LBP in DSAD. This question requires longitudinal studies with repeated biomarker assessments and neuropathological follow-up, which are beyond the scope of this study. Based on kinetic properties, it has been suggested that focal αSyn in AD may exhibit lower seeding activity.[6,9] Therefore, comparing kinetic profiles across different groups could enhance our understanding of these findings. Additionally, further studies should investigate the clinical impact of LBP on cognitive and motor symptoms, as well as explore relationships with other biomarkers, including neuroimaging.

In summary, this study highlights the interplay between LBP and DSAD, revealing a prevalence of αSyn seeding activity comparable to ADAD but lower than sAD, with a correlation between neocortical LBP and seeding activity in this population. These findings suggest that age-related dynamics and LBP progression in DSAD may be shaped by earlier amyloid spread and AD neuropathologic changes, emphasizing the need for further investigation into the mechanisms underlying LBP in genetically determined forms of AD.

AUTHOR CONTRIBUTIONS

Alexander Maximilian Bernhardt, Íñigo Rodríguez-Baz, Juan Fortea, and Johannes Levin contributed to the conception and design of

the study and had full access to the data used in the analyses and take responsibility for the integrity and accuracy of the data analysis. Alexander Maximilian Bernhardt, Íñigo Rodríguez-Baz, Daniel Alcolea, Juan Fortea, and Johannes Levin contributed to the analysis and interpretation of data. Alexander Maximilian Bernhardt, Íñigo Rodríguez-Baz, Iban Aldecoa, Javier Arranz, José Enrique Arriola-Infante, Lucia Maure-Blesa, Maria Carmona-Iragui, Sebastian Longen, Svenja Verena Trossbach, Armin Giese, Torsten Matthias, Bessy Benejam, Laura Videla, Laura del Hoyo Soriano, Isabel Barroeta, Susana Fernández, Lídia Vaqué-Alcázar, Mateus Rozalem Aranha, Alejandra O. Morcillo-Nieto, Georg Nübling, Olivia Wagemann, Anna Stockbauer, Mireia Tondo, Alexandre Bejanin, Alberto Lleó, Daniel Alcolea, Laura Molina-Porcel, Juan Fortea, and Johannes Levin contributed to the acquisition of data. All authors contributed to drafting and revising the manuscript for important intellectual content.

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CONFLICT OF INTEREST STATEMENT

MCI reported receiving personal fees for service on the advisory boards, speaker honoraria or educational activities from Esteve, Lilly, Neuraxpharm, Adium, and Roche diagnostics. MRA has provided paid consultancy for Veranex and is a partner and director of production at Masima—Soluções em Imagens Médicas LTDA. DA reported receiving personal fees for advisory board services and/or speaker honoraria from Fujirebio-Europe, Roche, Nutricia, Krka Farmacéutica, Lilly, Zambon S.A.U., Grifols, and Esteve, outside the submitted work. AL has served as a consultant or on advisory boards for Almirall, Fujirebio-Europe, Roche, Biogen, Grifols, NovoNordisk, Novartis, Eisai, Lilly, and Nutricia, outside the submitted work. LMP has provided consultancy services to Biogen. JF reported serving on the advisory boards, adjudication committees, or speaker honoraria from Roche, NovoNordisk, Esteve, Biogen, Laboratorios Carnot, Adamed, LMI, Novartis, Lundbeck, Roche, AC Immune, Alzheon, Zambon, Lilly, Spanish Neurological Society, T21 Research Society, Lumind foundation, Jérôme-Lejeune Foundation, Alzheimer's Association, NIH, USA, and Instituto de Salud Carlos III. JA reported receiving personal fees for service on speaker honoraria or educational activities from Lilly, Esteve, Fujirebio-Europe, and Roche diagnostics. DA, AL, and JF report holding a patent for markers of synaptopathy in neurodegenerative disease (licensed to ADx, EPI8382175.0). JL reports speaker fees from Bayer Vital, Biogen, EISAI, Merck, Roche, TEVA, and Zambon; consulting fees from Axon Neuroscience, EISAI, and Biogen; author fees from Thieme Medical Publishers and W. Kohlhammer GmbH Medical Publishers; and is inventor in the patent "Oral Phenylbutyrate for Treatment of Human 4-Repeat Tauopathies" (EP 23 156 122.6) filed by LMU Munich. In addition, he reports compensation for serving as chief medical officer for MODAG GmbH; is beneficiary of the phantom share program of MODAG GmbH; and is inventor in the patent "Pharmaceutical Composition and Methods of Use" (EP 22 159 408.8) filed by MODAG GmbH, all activities outside the submitted work. No other competing interests were reported. The author disclosures are available in the Supplementary Information.

DATA AVAILABILITY STATEMENT

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

CONSENT STATEMENT

Written informed consent was obtained from all participants or their legally authorized representatives.

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SUPPORTING INFORMATION

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

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