Received: 10 January 2025 | Revised: 14 May 2025 | Accepted: 20 May 2025

DOI: 10.1002/alz.70380

Alzheimer's & Dementia®
THE JOURNAL OF THE ALZHEIMER'S ASSOCIATION

RESEARCH ARTICLE

# Cargo of small extracellular vesicles from neuronal origin shows progression of dementia in individuals with Down syndrome

**Aurélie Ledreux**[1] | **Maria Carmona-Iragui**[2,3,4] | **Laura Videla**[2,3,4] | **Bessy Benejam**[4] | **Isabel Barroeta**[2,3] | **Alberto Lleó**[2,3] | **Daniel Alcolea**[2,3] | **Juan Fortea**[2,3,4]

[1]Department of Neurosurgery, School of Medicine, University of Colorado Anschutz Medical Campus, Aurora, Colorado, USA

[2]Sant Pau Memory Unit, Department of Neurology, Hospital de la Santa Creu i Sant Pau, Biomedical Research Institute Sant Pau, Universitat Autònoma de Barcelona, Barcelona, Spain

[3]Center of Biomedical Investigation Network for Neurodegenerative Diseases (CIBERNED), Madrid, Spain

[4]Barcelona Down Medical Center, Fundació Catalana Síndrome de Down, Carrer del Comte Borrell, Barcelona, Spain

**Correspondence**
Aurélie Ledreux, Department of Neurosurgery, School of Medicine, University of Colorado Anschutz Medical Campus, 12700 E. 19th Ave, Aurora, CO 80045.
Email: Aurelie.Ledreux@cuanschutz.edu

**Funding information**
Alzheimer's Association, Grant/Award Number: AARG-18-565696; Instituto de Salud Carlos III; Ministerio de Asuntos Económicos y Transformación Digital; Gobierno de España, Grant/Award Numbers: INT21/00073, PI20/01473, PI23/01786 to JF, PI18/00335, PI22/00785 to MCI; Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas Program; Fondo Europeo de Desarrollo Regional; Unión Europea; Una Manera de Hacer Europa; European Regional Development Fund/European Social Fund; National Institutes of Health, Grant/Award Numbers: R01 AG056850, R21 AG056974, R01 AG061566, R01 AG081394, R61AG066543; Fundación Tatiana Pérez de Guzmán el Bueno, Grant/Award Number: IIBSP-DOW-2020-151 to JF; European Union, Grant/Award Numbers: H2020-SC1-BHC-2018-2020 to JF, AARG-22-973966 to MCI; National Institute on Aging, Grant/Award Numbers: R01 AG061566, RF1 AG070153

## Abstract

**INTRODUCTION:** Individuals with Down syndrome (DS) are at an ultra-high risk of developing Alzheimer's disease (AD). Diagnosis of AD onset in people with DS can be challenging due to the variable degrees of intellectual disability and cognitive impairment among individuals.

**METHODS:** Plasma samples from individuals with DS diagnosed with AD dementia ($n = 33$), prodromal AD ($n = 31$), or cognitively stable ($n = 43$) were enriched for neuron-derived extracellular vesicles (NDEV) using immunocapture with the L1CAM antibody. We used single-molecule array technology to quantify amyloid-$\beta$ (A$\beta$) peptides, Tau, phosphorylated Tau, neurofilament light chain (NfL), and synaptosomal-associated protein 25 (SNAP25) across diagnostic groups.

**RESULTS:** NDEV levels of A$\beta$40, A$\beta$42, Tau, pTauT181, pTauT231, NfL, and SNAP25 were significantly higher in people with DS diagnosed with prodromal AD compared to those with no cognitive decline. Middle-aged or older women had higher levels of NDEV biomarkers compared to males.

**DISCUSSION:** NDEV biomarker levels can inform on the onset of AD in individuals with DS.

KEYWORDS
Alzheimer's disease, amyloid-beta, biomarker, dementia, Down syndrome, extracellular vesicle, neurofilament-light, phosphorylated Tau

## Highlights

- Diagnosis of Alzheimer's dementia in individuals with Down syndrome (DS)is challenging.

- Neuron-derived extracellular vesicles were enriched from plasma of adults with Down syndrome.
- Alzheimer's disease biomarkers were measured using single molecule array technology.
- NDEV biomarkers accurately predicted the prodromal stage of dementia in people with DS.

## 1 | BACKGROUND

Down syndrome (DS) is the most common aneuploidy and cause of intellectual disability of genetic origin, with an incidence of 1 in ≈700 babies in the United States.[1] Improved medical care has increased the life expectancy of individuals with DS, which now reaches 60 years of age,[2] making age-related comorbidities more prevalent in this population. The triplication of a segment or the entire chromosome 21 (Hsa21)[3] results in the overexpression of several genes believed to play a role in the development of Alzheimer's disease (AD) pathology and clinical manifestations of dementia[4] and puts individuals with DS at an ultra-high risk of developing AD at an earlier age than the general population.[5] The presence of the amyloid precursor protein (APP) gene on Hsa21 leads to the overproduction of amyloid-beta (A$\beta$), which plays a key role in DS-associated AD.[6] Other Hsa21 genes implicated in the dysregulation of Tau phosphorylation might also affect progression of AD pathology in DS.[7,8] Consequently, DS is now considered a form of genetically determined early-onset AD.[9]

Diagnosis of dementia in DS is tightly related to age,[10] with cognitive decline starting in the fifth decade and dementia diagnosed on average by 53 years of age.[11] Diagnosis of prodromal AD dementia (pDS) in individuals with DS can be challenging because of the variable degrees of cognitive impairment and intellectual disability among individuals.[12] Since early detection of AD is likely to affect the therapies and care that people with DS receive, much effort has been dedicated to advancing the diagnosis of prodromal AD using different biomarker methods, including neuroimaging.[13,14] However, with the notable exceptions of NfL and pTauT181,[15,16] blood biomarkers for the diagnosis of dementia in DS have shown low diagnostic performance, highlighting the need for the development of brain-specific non-invasive biomarkers that could also inform on disease processes.[17]

Extracellular vesicles (EVs) are lipid-delimited nanoparticles released by all cells in the body. They have gained attention because of their important functions in cell–cell communication.[18] Given their small size, EVs can diffuse from the central nervous system (CNS) to the peripheral circulation.[19] They can be enriched from the blood using immunocapture-based techniques, which makes them an attractive source for biomarker discovery.[20] EVs contain proteins, lipids, and nucleic acids that mirror the content of the cell they originate from, providing a snapshot of the homeostatic status of their cell of origin. Some EVs, referred to as microvesicles, are formed directly by budding of the plasma membrane, while others, often referred to as exosomes, are formed by the inward budding of the endosomal membrane, creating multivesicular bodies that then fuse with the plasma membrane to release exosomes in the extracellular space.[18] Small EVs enriched from blood, likely including both exosomes and small microvesicles, have recently emerged as a non-invasive promising source of biomarkers in cancer and neurodegenerative diseases.[21,22]

We previously demonstrated that *(i)* neuron-derived EVs (NDEVs) enriched from blood of individuals with DS had higher levels of A$\beta$42 peptides and phosphorylated Tau than age-matched non-DS controls, starting in childhood, and *(ii)* individuals with early or fully symptomatic dementia had higher levels of pTauS396, compared to individuals with no apparent dementia.[23] Building on these findings, we hypothesized that the cargo of NDEV would inform the progression of dementia in people with DS. To our knowledge, one previous study[24] has examined NDEV in DS, but this was done in the context of insulin signaling and mammalian target of rapamycin (mTOR) pathway in young individuals with DS. By including both common AD biomarkers and synaptosomal-associated protein 25 (SNAP25), our study offers a novel perspective on evaluating dementia progression in DS. Cerebrospinal fluid (CSF) levels of SNAP25 were shown to be a promising biomarker of synaptic degeneration in AD.[25,26] Our main objectives were to (1) compare the distribution of A$\beta$40, A$\beta$42, Tau, NfL, pTauT181, pTauT231, and SNAP25 concentrations in NDEVs in different clinical groups of individuals with DS; (2) correlate NDEV AD biomarker levels to CSF and/or plasma levels and cognitive measures; and (3) examine the predictive value of a combination of variables to detect dementia onset in a cohort of individuals with DS.

## 2 | MATERIAL AND METHODS

### 2.1 | Study participants

We included adults with DS (≥18 years) who had been evaluated at the Hospital de la Santa Creu i Sant Pau in Barcelona (Spain) through a population-based health plan designed to screen for AD dementia, which includes yearly neurological and neuropsychological assessments. The clinical status of each participant was determined following neuropsychological and neurological assessments.[27] The level of disability was categorized according to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition, as mild, moderate, severe, or profound intellectual disability, based on carers' reports of the individuals' best-ever level of functioning.[28] Each specialist conducted their evaluation independently, remaining blinded to each

other's evaluations and to the participants' biomarker status. Based on their consensus, participants were classified into the following clinical groups: asymptomatic (aDS) with no signs of AD; pDS with AD symptoms not meeting criteria for dementia; and dementia (dDS) with clear AD-related dementia.[27] Plasma and CSF levels of AD biomarkers were previously measured using single molecule array (Simoa; Quanterix) as described elsewhere.[27] Cambridge Cognitive Examination for Older Adults with Down syndrome (CAMCOG-DS, low scores associated with worse cognitive performance) and Dementia questionnaire for people with Learning Disabilities (DLD, high scores associated with worse cognition) scores were available for some participants included in this cohort.[28]

## 2.2 | Isolation of NDEVs from human plasma

Enrichment for NDEVs was performed in two sequential steps, consisting of polymer precipitation followed by immunocapture with L1CAM antibody as described previously.[29,30] Technical details can be found in the Supplemental Materials. All relevant data have been submitted to the EV-TRACK knowledgebase (ID EV250061).[31]

## 2.3 | NDEV characterization

The shape and size of NDEVs were verified using transmission electron microscopy (TEM). Briefly, 10 µL of NDEV suspension (in DPBS) was loaded onto a discharged grid and incubated for 5 min at room temperature. The excess liquid was then blotted with filter paper, and the grid immediately rinsed with 10 µL of deionized water. Thereafter, 10 µL of filtered 2% aqueous uranyl acetate was immediately added for negative staining. After full removal of the liquid from the grid, samples were air-dried for approximately 3 min. Samples were imaged at 80 kV on a Thermo Fisher Tecnai G2 12 BioTwin TEM equipped with a NS15B sCMOS AMT Camera.

NDEV size and concentration were assessed with two different methods. Fluorescent nanoparticle tracking analysis (NTA) with LM10 Nanosight apparatus (Malvern Instruments) was performed as described previously.[29] We also used the single-particle interferometric reflectance imaging sensor-based (SP-IRIS) analyzer (ExoView, NanoView Biosciences) to examine the tetraspanin distribution with the Human Tetraspanin Plasma kit assay kit according to the manufacturer's instructions. This assay captures EVs expressing CD63, CD81, CD9, with mouse IgG as negative control and CD41a as indicator of platelet-derived EVs.[32]

To demonstrate relatively specific enrichment for small EVs of neuronal origin, we compared biomarker levels in four plasma samples from healthy volunteers and corresponding NDEV preps. EV-specific markers, membrane-bound tetraspanin CD81, and luminal Alix were measured using commercial enzyme-linked immunosorbent assay (ELISA) kits (Cusabio; TX, USA). To show enrichment for relatively specific neuronal proteins, we measured, using Simoa technology on the SR-X instrument (Quanterix; MA, USA) the levels of Tau, UCH-L1, NfL, and SNAP25 in NDEV preps. We also measured the levels of plasma-abundant lipoprotein ApoB100 (Cat.# E08100h, Cusabio; TX, USA), as suggested by International Society for Extracellular Vesicles (ISEV) guidelines.[33]

### RESEARCH IN CONTEXT

1. **Systematic review**: The authors conducted a comprehensive review of the literature using both traditional sources (e.g., PubMed) and conference abstracts from the Alzheimer's Association International Conference. While numerous articles address plasma biomarkers of Alzheimer's disease (AD) in Down syndrome (DS), no studies have specifically examined biomarkers in neuron-derived extracellular vesicles (NDEV) in the context of AD progression in DS. All relevant previous work has been cited appropriately.
2. **Interpretation**: Our findings underscore the feasibility of using NDEV biomarkers to track dementia onset in individuals with DS. We provide evidence that the cargo of NDEV can serve as a source of brain-specific biomarkers, applicable not only to DS but also to other neurodegenerative conditions.
3. **Future directions**: Longitudinal studies leveraging NDEV and other brain-derived EV are needed to deepen our understanding of the pathological processes driving dementia onset in individuals with DS. Additionally, future work should prioritize validating the use of NDEV as a non-invasive approach to monitor brain-specific processes, with an emphasis on implementing standardization and interlaboratory validation experiments.

## 2.4 | Biomarker analysis

Levels of CD81 were measured in all NDEV preps using a colorimetric ELISA kit (EL004960Hu, Cusabio; TX, USA). NDEV biomarker levels were measured using ultrasensitive Simoa technology on the SR-X instrument (Quanterix; MA, USA). The human Neurology 3-plex A assay was used to measure Tau (N-terminal to mid-domain (R1) Tau), A$\beta$40, and A$\beta$42. NfL, SNAP25, pTauT181, and pTauT231 levels were measured using Quanterix singleplex assays on the SR-X platform, following manufacturer's instructions.

## 2.5 | Statistical analysis

Since group variances were significantly different, we used Welch's analysis of variance (ANOVA) followed by Dunnett's T3 *post hoc* multiple comparisons to test for group differences. Sex differences were

**TABLE 1** Cohort demographics.

| Parameter | Asymptomatic (aDS) | Prodromal Alzheimer's disease (pDS) | Alzheimer's disease dementia (dDS) | ANOVA *p*-value | Dunnett's T3 post hoc adjusted *p*-values |
|---|---|---|---|---|---|
| Sample size | 43 (40%) | 31 (29%) | 33 (31%) | – | |
| Age (years) | 40.4 [19.2–56.8] | 50.4 [44.2–59.7] | 53.7 [45.4–64.3] | *<0.001* | aDS-pDS: $p < 0.0001$ |
| | | | | | aDS-dDS: $p < 0.0001$ |
| | | | | | pDS-dDS: $p = 0.036$ |
| Biological sex | | | | | |
| Male | 29 (67%) | 15 (48%) | 16 (48%) | 0.152 | |
| Female | 14 (33%) | 16 (52%) | 17 (52%) | | |
| Intellectual disability | | | | | |
| Mild | 13 (30%) | 4 (13%) | 3 (9%) | *0.032* | |
| Moderate | 21 (49%) | 13 (42%) | 24 (73%) | | |
| Severe or profound | 9 (21%) | 14 (45%) | 6 (18%) | | |
| CAMCOG-DS score | 73 [31–101] *32 | 47 [26–73] *10 | 40 [2–72] *16 | *<0.001* | aDS-pDS: $p = 0.002$ |
| | | | | | aDS-dDS: $p < 0.0001$ |
| DLD score | 18 [0–55] *34 | 32 [13–69] *13 | 48 [6–75] *21 | *<0.001* | aDS-pDS: $p = 0.022$ |
| | | | | | aDS-dDS: $p < 0.001$ |
| | | | | | pDS-dDS: $p = 0.034$ |

*Note*: Data are presented as *n* (%) or mean [range]. CAMCOG-DS and DLD scores were available only for some individuals in each group (number of individuals indicated after the asterisk *). Group differences were tested with Chi-square test for sex, Kruskal–Wallis test for intellectual disability and one-way ANOVA for continuous variables (age, CAMCOG-DS and DLD scores). For continuous variables, significant adjusted pairwise comparisons *p*-values (Dunnett's T3) are presented.

Abbreviations: ANOVA, analysis of variance; CAMCOG-DS, Cambridge Cognitive Examination for Older adults with Down syndrome; DLD, Dementia questionnaire for people with Learning Disabilities.

examined with Welch's *t*-test and two-way ANOVA with sex and diagnosis (or age group) as independent variables. Correlation analysis between NDEV, plasma, and CSF, and cognitive measures was carried out using Spearman's correlations. To assess which biomarker or combination of biomarkers would best predict the dementia status, we used backward stepwise logistic regression models using all NDEV biomarker measures, age, and level of intellectual disability to calculate predicted values that were used to build receiver operating characteristics (ROC) curves. NDEV SNAP25 levels were not included in the logistic regression analysis as measurements were available for a limited number of subjects. Backward stepwise logistic regression results were confirmed by running logistic regressions on all possible combinations of predictive variables using ChatGPT (OpenAI). Statistical analyses were performed with SPSS (v28), and graphs were prepared with GraphPad Prism (v10).

## 3 | RESULTS

### 3.1 | Participants demographics

We included NDEVs enriched from 107 plasma samples from individuals with DS. At the time of blood collection, 43 were asymptomatic (aDS), 31 were diagnosed with pDS, and 33 with AD dementia (dDS, Table 1). As expected, participants asymptomatic for AD were significantly younger than those in the pDS and dDS groups ($p < 0.001$), although the age ranges showed some overlap between groups. There were significant differences in the distribution of intellectual disability among groups ($p = 0.032$). CAMCOG-DS and DLD scores, available only for some participants, were significantly different between groups (Table 1).

### 3.2 | Characterization of NDEVs

The analysis of intact NDEVs using fluorescent NTA revealed an enrichment of EVs in the expected size range, with concentrations ranging from $10^8$–$10^9$ particles/mL, and an average size of 102 nm (Figure S1A). Transmission electron microscopy revealed EVs within the expected size range (Figure S1B). Further, using the SP-IRIS platform, we found NDEV concentrations ranging from $1 \times 10^7$ EV/mL to $1.2 \times 10^8$ EV/mL, with a median size of ≈50 nm (Figure S1C), as typically observed on this platform.[32] A majority of NDEV were captured by CD9, followed by CD63 and CD81 (Figure S2A). Colocalization analysis (Figure S2B) demonstrated an enrichment of double-positive NDEV captured on the tetraspanin chips compared to the CD41a and MIgG chips.

Using commercial ELISA kits, we found that CD81 and Alix levels were significantly elevated (Figure S3A,B) while levels of apolipoprotein B (ApoB) were decreased 200-fold (Figure S3C) in the NDEV

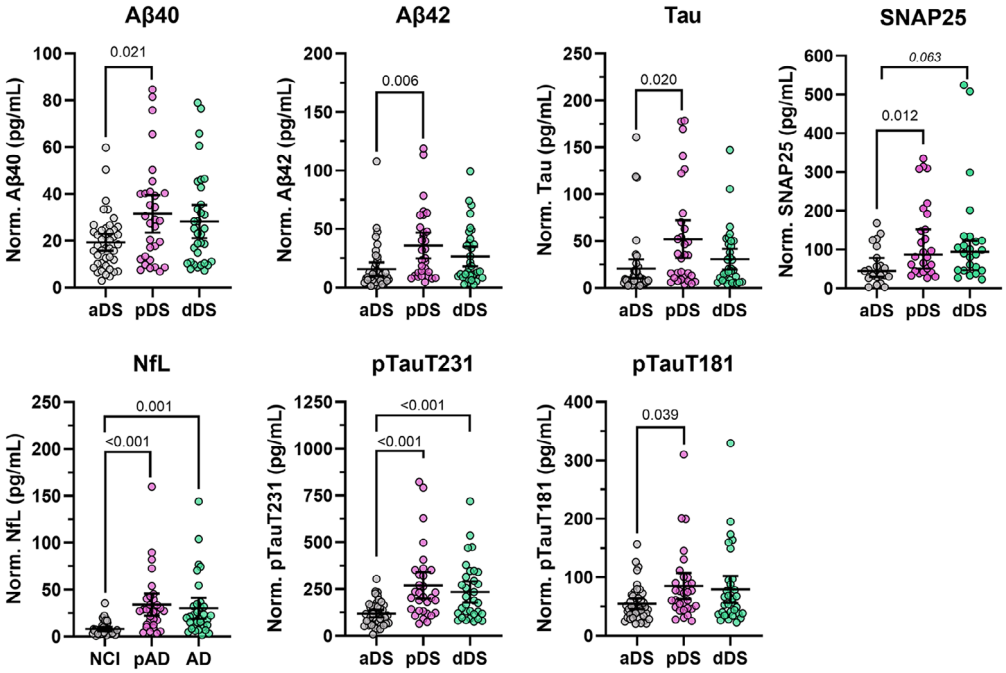


**FIGURE 1** Biomarker levels (black bars show mean ± 95% CI) in neuron-derived EVs. Group differences were tested with Welch's ANOVA test, followed by Dunnett's T3 multiple comparisons test. *p*-Values were adjusted for multiple comparisons. aDS, asymptomatic Down syndrome; ANOVA, analysis of variance; CI, confidence interval; dDS, Alzheimer's disease dementia Down syndrome; EV, extracellular vesicle; pDS, prodromal Alzheimer's disease Down syndrome.

preps compared to matching plasma samples, demonstrating a relatively specific enrichment for EVs in our preparations. Next, to confirm enrichment for neuronal EVs in our preparations, we measured levels of specific neuronal biomarkers (UCH-L1, NfL, SNAP25, and Tau) as well as GFAP (glial fibrillary acidic protein, an astrocyte-specific marker) in the NDEVs preps and matching plasma samples. All four neuronal proteins were measurable to some extent in plasma samples using the Simoa technology (Quanterix; MA, USA) (Figure S3E,H). However, levels of the neuron-specific proteins were between 5 and 2500 times higher in NDEV preps than in matching plasma samples, confirming a specific enrichment of EVs from neuronal origin.

## 3.3 | AD biomarker levels in NDEVs

The levels of tetraspanin CD81 were not significantly different between diagnosis groups ($p = 0.196$, Figure S4). The mean value of CD81 levels in each diagnosis group was set to 1.00, and the relative values for each sample were used to normalize the biomarker levels.[34] We examined the effects of dementia progression on the cargo of NDEV from individuals with DS by measuring the levels of seven biomarkers indicative of neurodegenerative processes. NDEV levels of Aβ40, Aβ42, Tau, pTauT231, pTauT181, SNAP25, and NfL were significantly higher in individuals with pDS, compared to asymptomatic participants with DS (Table S1 and Figure 1). Interestingly, in NDEVs from individuals with clinically diagnosed AD, the levels of biomarkers were stable or even slightly decreased compared to individuals with prodromal AD, which resulted in non-significant group differences between the pDS and dDS groups (Figure 1). We found no significant differences based on ID levels (Figure S5). Lastly, we found that only NDEV NfL levels correlated with age (Spearman rho = 0.318, $p = 0.001$, Figure S6).

## 3.4 | Effects of biological sex on NDEV biomarker levels

We previously reported[23] that men with DS had higher levels of pTauT181 than women with DS, which prompted us to examine sex differences in this study. We found that women had significantly higher NDEV biomarker levels than men (all $p < 0.05$, Welch's *t*-test, Figure 2A), except for pTauT181 and SNAP25 (not shown). We further investigated this across diagnosis groups using two-way ANOVA (sex × diagnosis). The main effect of diagnosis was statistically significant for all biomarkers (all $p < 0.03$), except for SNAP25. Using pairwise comparisons, we found no sex difference in the asymptomatic group. However, in the dDS group, women had significantly higher levels of Aβ42, NfL, pTauT231, and pTauT181 than men (all $p \leq 0.05$, Figure 2B). Further, in the pDS group, women tended to have higher levels of pTauT231 compared to men ($p = 0.09$, Figure 2B). Overall, these results point to sex differences emerging in symptomatic stages of the disease. However, cohort demographics show that age is not always a good indicator of clinical diagnosis (see Table 1). To further narrow down the age window during which the difference between men and women arises, we stratified by age groups, with age groups being defined as less than 43 years of age, between 43 and 49, and older than 50 years of age.

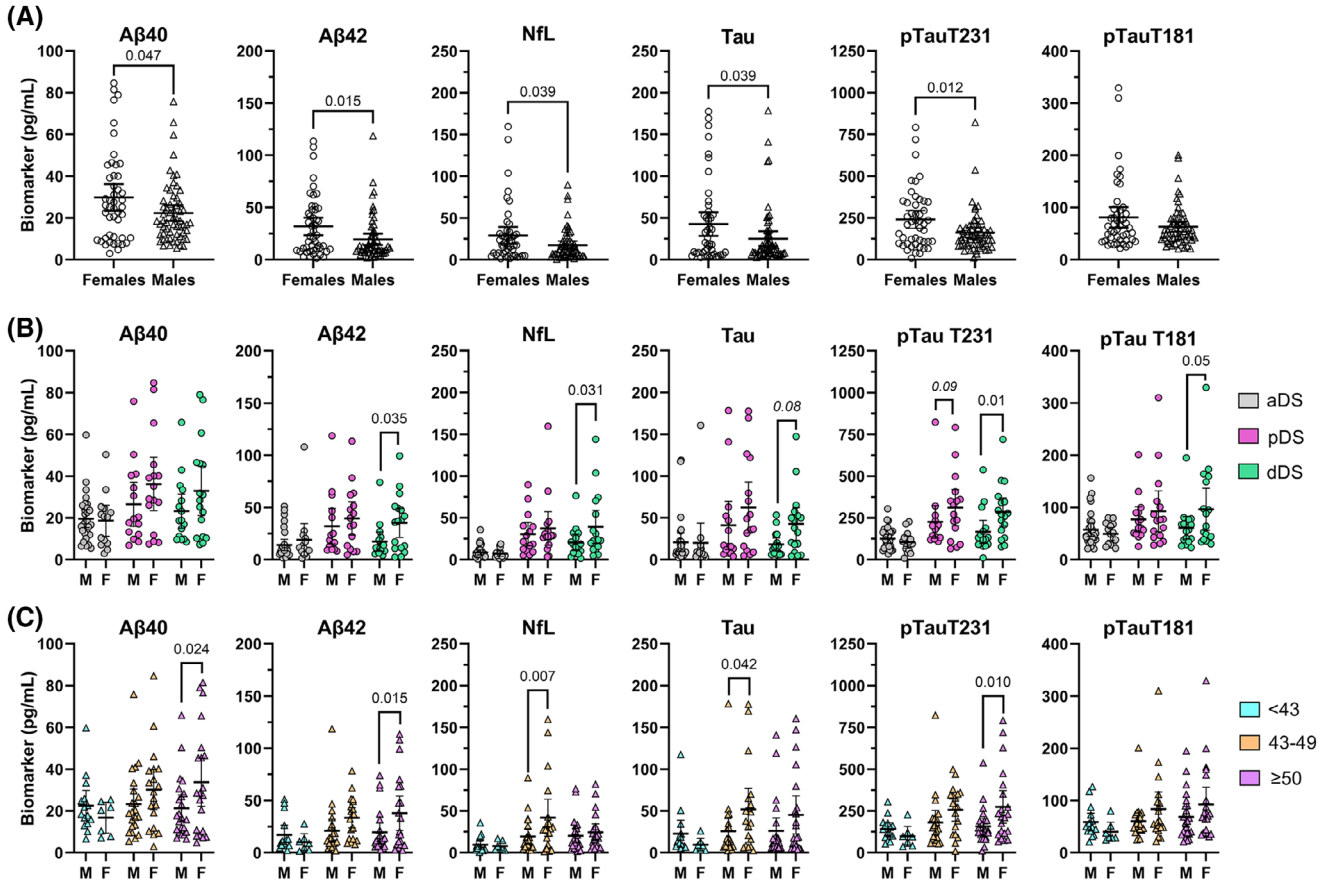


**FIGURE 2** Biological sex differences in AD biomarker levels in neuron-derived EVs in the entire cohort (A), by diagnosis group (B), and by age group (C). Data are presented as mean ± 95% confidence intervals. (A) Women had significantly higher levels of AD biomarkers (except pTauT181) in NDEVs (Welch's *t*-test). (B) Women in the AD diagnosis group (dDS) had significantly higher levels of all biomarkers (except Aβ40) compared to men. (C) When the cohort was divided based on age groups (younger than 43, between 43 and 49, and 50 and older), women older than 50 years had significantly higher levels of Aβ40, Aβ42, and pTauT231, whereas 43- to 49-year-old women had higher levels of Tau and NfL, in contrast to what was observed with diagnosis groups. Aβ, amyloid-beta; AD, Alzheimer's disease; EV, extracellular vesicle; NDEV, neuron-derived extracellular vesicle; NfL, neurofilament light chain.

We found that women had significantly higher levels of Aβ40 and Aβ42 than men in the older group (> 50 years) while their levels of Tau and NfL were highest between 43 and 49 years of age (Figure 2C). Overall, sex differences appeared similarly distributed between age and clinical diagnosis only for Aβ42 and pT231 (Figure 2B,C).

## 3.5 | NDEV biomarker levels correlate with CSF and blood biomarkers

We next examined whether NDEV and plasma or CSF biomarker levels correlated, hypothesizing that CSF and NDEV levels, because of their CNS origin, would correlate more strongly than plasma and NDEV levels. NDEV NfL levels correlated positively with CSF NfL, Tau, and pTauT231, as well as with plasma levels of pTauT231, NfL, and pTauT181 (Figure 3). NDEV Aβ42 levels correlated negatively with CSF Aβ42 levels only marginally, but positively with CSF Tau and pTau as well as with plasma pTauT181 levels. NDEV Tau levels were positively correlated with CSF Tau and Aβ42 levels, as well as with plasma pT181 levels. NDEV pTauT231 levels were positively correlated with CSF Aβ42, NfL, Tau, and pT231 levels. NDEV pTauT181 and SNAP25 levels did not correlate with other CSF or plasma measures.

## 3.6 | Relationships between NDEV biomarker levels and cognitive measures

CAMCOG-DS and DLD scores were available for more than half of the cohort participants, predominantly in the asymptomatic group (Table 1). We found that NDEV NfL, and SNAP25 levels were negatively correlated with CAMCOG-DS scores across the entire cohort (Figure S7A,B). Further, we found positive significant correlations between NDEV levels of Tau, Aβ42, and NfL with DLD scores (rho = 0.247, $p = 0.043$; rho = 0.277, $p = 0.022$; and rho = 0.430, $p < 0.001$, respectively; Figure S7C–E).

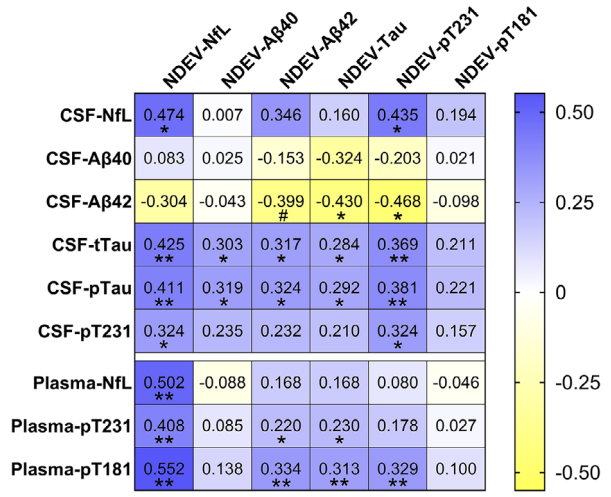


**FIGURE 3** Spearman correlations heatmap. Spearman rho-values are presented with *p*-values (#$p < 0.07$; *$p < 0.05$, **$p < 0.01$). Correlations were run using partial datasets for CSF and plasma biomarkers. CSF biomarker levels were available for 22 participants (A$\beta$40, A$\beta$42, and NfL) and 55 participants (tTau, pTau, and pT231). Plasma biomarker levels were available for 59, 90, and 71 participants, respectively, for NfL, pT231, and pT181. NDEV levels of SNAP25 (not shown) did not correlate with any other measures. A$\beta$, amyloid-beta; CSF, cerebrospinal fluid; NDEV, neuron-derived extracellular vesicle; NfL, neurofilament light chain.

## 3.7 | Onset of dementia in DS is predicted by age and increase in NDEV biomarker levels

We used a backward stepwise logistic regression model to examine which combination of the six NDEV biomarkers, along with age and ID levels, would best predict the onset of AD in DS. A model including the variables age, NfL, and pTauT231 levels was the most predictive of the progression from aDS to pDS, giving an area under the ROC curve (AUC) of 0.96 (95% confidence interval [CI] 0.92–0.99) (Table 2 and Figure 4). When examining the transition from pDS to dDS dementia, only the variables age and ID levels stood out as the most predictive (AUC = 0.76 [95% CI 0.65–0.88]), with no NDEV biomarker remaining in the model. When comparing aDS versus combined pDS and dDS groups, age, NfL, pT231, and A$\beta$42 remain in the model, producing an AUC of 0.96 [95% CI 0.92–0.99]. These findings were further supported by running logistic regressions on each possible combination of predictive variables (see Table S2).

Lastly, we examined correlations between NDEV biomarker levels for each diagnosis group. Overall, with the exception of NfL, stronger positive Spearman correlations were observed in the pDS and dDS groups compared to the aDS group (Figure S8), potentially indicating dynamic changes associated with compensatory mechanisms aimed at clearing toxic proteins from neurons.

## 4 | DISCUSSION

Detection of AD dementia onset in people with DS is challenging due to variable levels of intellectual disability, making it particularly diffi-

**TABLE 2** Results of backward stepwise logistic regression models.

| aDS vs. pDS | | | | pDS vs. dDS | | | | aDS vs. dDS | | | | aDS vs. (pDS + dDS) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Variable | OR | 95% CI | *p*-Value | Variable | OR | 95% CI | *p*-Value | Variable | OR | 95% CI | *p*-Value | Variable | OR | 95% CI | *p*-Value |
| NfL | 1.172 | 1.06–1.29 | 0.002 | ID: moderate | 5.070 | 1.55–16.6 | 0.007 | NfL | 1.201 | 1.04–1.38 | 0.010 | NfL | 1.179 | 1.06–1.31 | 0.002 |
| pTauT231 | 1.011 | 1.00–1.02 | 0.048 | Age | 1.174 | 1.04–1.33 | 0.010 | A$\beta$40 | 1.096 | 1.01–1.19 | 0.035 | A$\beta$42 | 0.963 | 0.92–1.00 | 0.079 |
| Age | 1.272 | 1.08–1.50 | 0.004 | | | | | A$\beta$42 | 0.947 | 0.90–1.00 | 0.051 | pTauT231 | 1.015 | 1.00–1.03 | 0.023 |
| | | | | | | | | Age | 1.392 | 1.13–1.72 | 0.002 | Age | 1.335 | 1.15–1.55 | <0.001 |

*Note:* Variables that are significantly contributing to the prediction of dementia status are presented.
Abbreviations: 95% CI, 95% confidence interval; aDS, asymptomatic; dDS, AD dementia; ID, intellectual disability; NfL, neurofilament light chain; OR, odds ratios; pDS, prodromal AD.

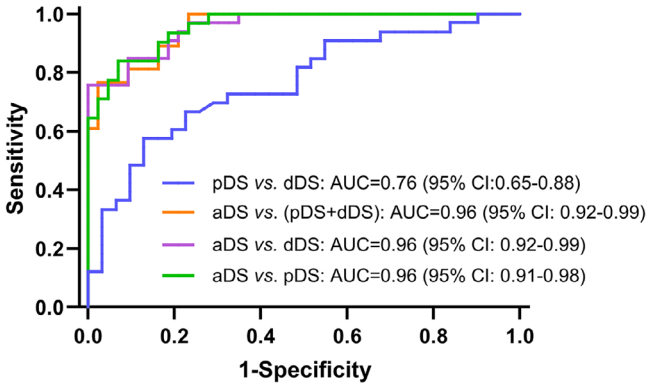


**FIGURE 4** Receiver operating characteristics (ROC) curves comparing the asymptomatic (aDS) group with prodromal AD dementia (pDS) group (green curve), with the dementia (dDS) (purple curve) group, and with the combined pDS and dDS groups (orange curve). The blue curve depicts the ROC curve comparing the pDS to the dDS group. Area under the ROC curves (AUC) and 95% confidence intervals (95% CI) are presented for each curve. The ROC curves correspond to the best backward stepwise logistic regression model for each comparison.

cult to diagnose the onset of AD symptomatology in this population. We report that the levels of Aβ40, Aβ42, Tau, pTauT231, pTauT181, SNAP25, and NfL in NDEVs enriched from plasma are elevated in symptomatic AD stages compared to cognitively stable (CS) individuals with DS. These findings add to our previous study in which we reported that individuals with DS had increased NDEV levels of Aβ42 and pTauS396 compared to non-DS cases.[23] Novel findings of this study include that females had higher biomarker levels than men in symptomatic patients and in the older participants. Lastly, we demonstrated an excellent diagnostic performance of NDEV biomarker levels of NfL and pTauT231 in combination with age to distinguish aDS from pDS cases with an AUC of 96%.

While there has been some debate around the use of the L1CAM antibody to enrich plasma/serum samples for EVs from neuronal origin,[35] recent studies have unambiguously demonstrated the presence of the ectodomain of L1CAM on neuronal EVs.[36,37] In our validation experiments, we observed several-fold increases in NDEV levels of specific neuronal proteins such as Tau, SNAP25, and UCH-L1 and a significant decrease in astrocytic GFAP, when compared to matching plasma samples, supporting the idea of a relatively specific enrichment for neuronal EVs. Interestingly, we did not find any differences in terms of levels of CD81 between the diagnostic groups, suggesting that similar numbers of NDEVs were released into plasma in all participants. Although we did not include a non-DS control group in this study, CD81 levels measured in NDEV from another non-DS cohort were lower,[38] supporting the observation that individuals with DS may release increased numbers of NDEVs.[39] Nonetheless, future studies should include other methods to quantify NDEV abundance, such as microfluidic resistive pulse sensing or nanoflow cytometry.[40]

A major finding of this study is the significant increase in NDEV levels of Aβ40, Aβ42, Tau, pTauT181, pTauT231, SNAP25, and NfL between CS individuals with DS and those diagnosed with prodromal AD, with percent increases ranging from 55% (pTauT181) to 317% (NfL). Detection of AD pathology in living individuals currently relies on CSF biomarkers or neuroimaging, which provide insights into neuropathological events and can help diagnose AD. However, these techniques require a high level of expertise, remain invasive, expensive, and are not well-suited for the inclusion of people with DS in clinical trials.[2] In this context, plasma biomarkers have gained traction as a minimally invasive option for AD diagnosis in DS. While plasma levels of Aβ40, Aβ42, and Tau showed low diagnostic accuracy,[41] plasma NfL, albeit increased with age and not specific to AD, was found in distinct cohorts to have good diagnostic performance to detect AD in adults with DS.[15,42,43] In a large cohort of adults with DS, plasma pTauT181 was shown to have good diagnostic accuracy (AUC = 0.92) for differentiating CS individuals from those with dementia but did not perform as well for distinguishing prodromal AD from CS individuals (AUC = 0.80).[16] However, plasma levels of pTauT181 can be confounded by comorbidities such as chronic kidney disease and hypertension,[44] which appear to be prevalent in individuals with DS,[45] highlighting the need for more brain-specific biomarkers. Future studies should examine NDEV levels of ptau217 or pTau212 as they were shown to be accurate plasma biomarkers of both Tau and Aβ pathology in DS.[46,47]

Findings from CSF biomarker measures showed significant differences in Aβ42, Tau, and pTauT181 levels between asymptomatic and symptomatic AD stages in DS,[27,28,48] but consistent with our findings in NDEV, showed no differences between prodromal AD and AD. Between 50 and 55 years of age, many individuals with DS will transition from no cognitive impairment (NCI) to symptomatic AD.[9,10] Neuropathological studies have found that neuronal loss reaches its highest levels (about 30%) between ages 51 and 59, while the percentage of neurons with neurofibrillary tangles and the amyloid load both appear stable in individuals with DS older than 51 years.[49] Our findings suggest that the number of EVs released remains consistent between diagnostic groups; however, this conclusion is limited by the reliance on CD81 levels as an indicator of the quantity of EVs released. When considered alongside the drastic neuronal loss, this observation aligns with studies pointing toward increased EV production and release with age, perhaps as a compensatory mechanism to clear toxic, unwanted materials in DS.[39,50] Our study significantly adds to this by suggesting that as the disease progresses, fewer neurons release similar levels of EVs, which cargo contains more AD-related proteins. This is also supported by the stronger positive correlations between NDEV biomarker levels in the pDS group, and to a lesser extent in the dDS stage of disease progression.

A limited number of studies have examined NDEV biomarkers in the context of sporadic AD. Conflicting results have been reported using immunocapture of NDEVs with the NCAM1 antibody. While Jia et al.[34] reported stepwise significant increases from control (NCI) to mild cognitive impairment (MCI) to AD participants in NDEV levels of Aβ42, Tau, and pTauT181, a recent study by Boyer and colleagues[51] found no difference between control and AD groups. Using the L1CAM antibody, Winston et al.[52] reported higher increases in NDEV Aβ42, pTauT181, and pTauS396 levels in adults who transitioned to AD within 3 years

compared to participants with stable MCI, but, consistent with our findings, no difference with the AD group.

In the non-DS population, women are at higher risk of developing AD than men, and autopsy studies reported women to have greater Tau pathology than men.[53] In DS, few studies have examined sex differences in blood biomarkers. Since women with DS experience menopause around 45 years of age,[54] it can be hypothesized that the drop in estrogen levels would correspond to increased AD-related pathology. Indeed, a strong association between lower levels of estrogen and higher risk for AD has been observed in DS.[55] In a recent large cohort study, Iulita and colleagues[56] found comparable levels of AD-related plasma and CSF biomarkers between males and females. However, when stratified by age, females between 40 and 50 years of age had higher levels of NfL and pTauT181.[56] Our findings expand these observations, as we found higher levels of AD-related NDEV biomarkers in females compared to males, and further found that neuronal damage markers NfL and Tau were higher in females between 43 and 49 years of age, while A$\beta$ levels were higher in females older than 50 years.

One obvious limitation of our study is the relatively small number of individuals in each diagnosis group and the cross-sectional nature of the sampling. APOE status was not available for all participants, preventing its inclusion in the analysis. Since neuroinflammation is elevated in individuals with DS,[57] assessing longitudinal changes in AD markers and neuroinflammatory markers in NDEVs would provide insights into the neuropathological trajectories in alive individuals with DS. Our results show that biomarkers in NDEVs enriched from plasma can be used to monitor AD progression and could complement neuroimaging or CSF studies to monitor effects of treatment therapies in DS.

## ACKNOWLEDGMENTS

We are grateful to the participants with Down syndrome and their caregivers who participated in this study for their time and efforts in supporting this research. The authors acknowledge the assistance of Anne Vielle and the Electron Microscopy Core Facility at CU Anschutz. The authors wish to thank Dr. Ann-Charlotte Granholm for her comments on this manuscript. This work was supported by grants from the Alzheimer's Association (AARG-18-565696 to AL) and from NIH/NIA (R01 AG061566 and RF1 AG070153 to AL). This study was also funded by the Instituto de Salud Carlos III (Ministerio de Asuntos Económicos y Transformación Digital, Gobierno de España) through the projects INT21/00073, PI20/01473 and PI23/01786 to JF, PI18/00335 and PI22/00785 to MCI) and the Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas Program 1, partly jointly funded by Fondo Europeo de Desarrollo Regional, Unión Europea, Una Manera de Hacer Europa, and cofunded by the European Regional Development Fund/European Social Fund (ERDF/ESF), 'A way to make Europe'/'Investing in your future'. This work was also supported by the National Institutes of Health grants (R01 AG056850; R21 AG056974, R01 AG061566, R01 AG081394 and R61AG066543 to JF. It was also supported by Fundación Tatiana Pérez de Guzmán el Bueno (IIBSP-DOW-2020-151 to JF), Horizon 2020–Research and Innovation Framework Programme from the European Union (H2020-SC1-BHC-2018-2020 to JF) and Alzheimer's Association (AARG-22-973966 to MCI).

## CONFLICT OF INTEREST STATEMENT

J.F. reported receiving personal fees for service on the advisory boards, adjudication committees or speaker honoraria from AC Immune, Adamed, Alzheon, Biogen, Eisai, Esteve, Fujirebio, Ionis, Laboratorios Carnot, Life Molecular Imaging, Lilly, Lundbeck, Novo Nordisk, Perha, Roche, Zambón and outside the submitted work. J.F. reports holding a patent for markers of synaptopathy in neurodegenerative disease (licensed to ADx, EPI8382175.0). M.C.I. reports receiving personal fees for service on the advisory boards, speaker honoraria or educational activities from Esteve, Lilly, Neuraxpharm, Adium and Roche diagnostics. D.A. participated in advisory boards from Fujirebio-Europe, Roche Diagnostics, Grifols S.A. and Lilly, and received speaker honoraria from Fujirebio-Europe, Roche Diagnostics, Nutricia, Krka Farmacéutica S.L., Zambon S.A.U., Neuraxpharm, Alter Medica, Lilly and Esteve Pharmaceuticals S.A. D.A. declares a filed patent application (WO2019175379 A1 Markers of synaptopathy in neurodegenerative disease). Al.L. reported receiving consulting fees from Eisai, Esteve, Fujirebio-Europe, Roche, Grifols S.A. and Lilly. A.L, B.B. and L.V. do not have anything to disclose. Author disclosures are available in the Supporting Information.

## CONSENT STATEMENT

The study was approved by the Ethical review Board of the Sant Pau Research Institute, following the standards for medical research in humans recommended by the Declaration of Helsinki. All participants or their legally authorized representatives gave written informed consent before enrollment.

## ORCID

*Aurélie Ledreux* https://orcid.org/0000-0001-6232-7672

## REFERENCES

1. Capone GT, Chicoine B, Bulova P, et al. Co-occurring medical conditions in adults with Down syndrome: a systematic review toward the development of health care guidelines. *Am J Med Genet A*. 2018;176:116-133. doi:10.1002/ajmg.a.38512
2. Sukreet S, Rafii MS, Rissman RA. From understanding to action: exploring molecular connections of Down syndrome to Alzheimer's disease for targeted therapeutic approach. *Alzheimers Dement (Amst)*. 2024;16:e12580. doi:10.1002/dad2.12580
3. Ballard C, Mobley W, Hardy J, Williams G, Corbett A. Dementia in Down's syndrome. *Lancet Neurol*. 2016;15:622-636. doi:10.1016/S1474-4422(16)00063-6
4. Gomez W, Morales R, Maracaja-Coutinho V, Parra V, Nassif M. Down syndrome and Alzheimer's disease: common molecular traits beyond the amyloid precursor protein. *Aging*. 2020;12:1011-1033. doi:10.18632/aging.102677
5. Iulita MF, Garzón Chavez D, Klitgaard Christensen M, et al. Association of Alzheimer disease with life expectancy in people with Down syndrome. *JAMA Netw Open*. 2022;5(5):e2212910. doi:10.1001/jamanetworkopen.2022.12910

6. Rumble B, Retallack R, Hilbich C, et al. Amyloid A4 protein and its precursor in Down's syndrome and Alzheimer's disease. *N Engl J Med*. 1989;320:1446-1452. doi:10.1056/nejm198906013202203
7. Wong H, Levenga J, Cain P, Rothermel B, Klann E, Hoeffer C. RCAN1 overexpression promotes age-dependent mitochondrial dysregulation related to neurodegeneration in Alzheimer's disease. *Acta Neuropathol*. 2015;130:829-843. doi:10.1007/s00401-015-1499-8
8. Wegiel J, Gong CX, Hwang YW. The role of DYRK1A in neurodegenerative diseases. *FEBS J*. 2011;278:236-245. doi:10.1111/j.1742-4658.2010.07955.x
9. Fortea J, Zaman SH, Hartley S, Rafii MS, Head E, Carmona-Iragui M. Alzheimer's disease associated with Down syndrome: a genetic form of dementia. *Lancet Neurol*. 2021;20:930-942. doi:10.1016/S1474-4422(21)00245-3
10. Larsen FK, Baksh RA, McGlinchey E, et al. Age of Alzheimer's disease diagnosis in people with Down syndrome and associated factors: results from the Horizon 21 European Down syndrome consortium. *Alzheimers Dement*. 2024;20:3270-3280. doi:10.1002/alz.13779
11. Strydom A, Coppus A, Blesa R, et al. Alzheimer's disease in Down syndrome: an overlooked population for prevention trials. *Alzheimers Dement*. 2018;4:703-713. doi:10.1016/j.trci.2018.10.006
12. Shimizu E, Goto-Hirano K, Motoi Y, Arai M, Hattori N. Symptoms and age of prodromal Alzheimer's disease in Down syndrome: a systematic review and meta-analysis. *Neurol Sci*. 2024;45:2445-2460. doi:10.1007/s10072-023-07292-9
13. Handen BL, Christian BT. *The Neurobiology of Aging and Alzheimer Disease in Down Syndrome*. Elsevier; 2021:173-192. doi:10.1016/B978-0-12-818845-3.00013-X
14. Abrahamson EE, Head E, Lott IT, et al. Neuropathological correlates of amyloid PET imaging in Down syndrome. *Dev Neurobiol*. 2019;79:750-766. doi:10.1002/dneu.22713
15. Carmona-Iragui M, Alcolea D, Barroeta I, et al. Diagnostic and prognostic performance and longitudinal changes in plasma neurofilament light chain concentrations in adults with Down syndrome: a cohort study. *Lancet Neurol*. 2021;20:605-614. doi:10.1016/S1474-4422(21)00129-0
16. Lleó A, Zetterberg H, Pegueroles J, et al. Phosphorylated tau181 in plasma as a potential biomarker for Alzheimer's disease in adults with Down syndrome. *Nat Commun*. 2021;12:4304. doi:10.1038/s41467-021-24319-x
17. Iulita MF, Granholm AC, Carmona-Iragui M, Hamlett ED, Fortea J, Ledreux A. Fluid biomarkers for Alzheimer's disease in Down syndrome: current status and novel trends. *The Neurobiology of Aging and Alzheimer Disease in Down Syndrome*. Elsevier; 2021:97-128. doi:10.1016/B978-0-12-818845-3.00010-4
18. Gurung S, Perocheau D, Touramanidou L, Baruteau J. The exosome journey: from biogenesis to uptake and intracellular signalling. *Cell Commun Signaling*. 2021;19:47. doi:10.1186/s12964-021-00730-1
19. Banks WA, Sharma P, Bullock KM, Hansen KM, Ludwig N, Whiteside TL. Transport of extracellular vesicles across the blood-brain barrier: brain pharmacokinetics and effects of inflammation. *Int J Mol Sci*. 2020;21:1-21. doi:10.3390/ijms21124407
20. Badhwar AP, Hirschberg Y, Valle-Tamayo N, et al. Assessment of brain-derived extracellular vesicle enrichment for blood biomarker analysis in age-related neurodegenerative diseases: an international overview. *Alzheimers Dement*. 2024;20:4411-4422. doi:10.1002/alz.13823
21. Yu D, Li Y, Wang M, et al. Exosomes as a new frontier of cancer liquid biopsy. *Mol Cancer*. 2022;21:56. doi:10.1186/s12943-022-01509-9
22. Younas N, Fernandez Flores LC, Hopfner F, Höglinger GU, Zerr I. A new paradigm for diagnosis of neurodegenerative diseases: peripheral exosomes of brain origin. *Transl Neurodegener*. 2022;11:28. doi:10.1186/s40035-022-00301-5
23. Hamlett ED, Goetzl EJ, Ledreux A, et al. Neuronal exosomes reveal Alzheimer's disease biomarkers in Down syndrome. *Alzheimers Dement*. 2017;13:541-549. doi:10.1016/j.jalz.2016.08.012
24. Perluigi M, Picca A, Montanari E, et al. Aberrant crosstalk between insulin signaling and mTOR in young Down syndrome individuals revealed by neuronal-derived extracellular vesicles. *Alzheimers Dement*. 2022;18:1498-1510. doi:10.1002/alz.12499
25. Brinkmalm A, Brinkmalm G, Honer WG, et al. SNAP-25 is a promising novel cerebrospinal fluid biomarker for synapse degeneration in Alzheimer's disease. *Mol Neurodegener*. 2014;9:53. doi:10.1186/1750-1326-9-53
26. Liu Q, Liu H, Zhang S, Yang Q, Shen L, Jiao B. Cerebrospinal fluid synaptosomal-associated protein 25 levels in patients with Alzheimer's disease: a meta-analysis. *J Alzheimers Dis*. 2022;89:121-132. doi:10.3233/JAD-215696
27. Fortea J, Vilaplana E, Carmona-Iragui M, et al. Clinical and biomarker changes of Alzheimer's disease in adults with Down syndrome: a cross-sectional study. *The Lancet*. 2020;395:1988-1997. doi:10.1016/S0140-6736(20)30689-9
28. Fortea J, Carmona-Iragui M, Benejam B, et al. Plasma and CSF biomarkers for the diagnosis of Alzheimer's disease in adults with Down syndrome: a cross-sectional study. *Lancet Neurol*. 2018;17:860-869. doi:10.1016/S1474-4422(18)30285-0
29. Ledreux A, Thomas S, Hamlett ED, et al. Small neuron-derived extracellular vesicles from individuals with Down syndrome propagate tau pathology in the wildtype mouse brain. *J Clin Med*. 2021;10:3931. doi:10.3390/jcm10173931
30. Kapogiannis D, Mustapic M, Shardell MD, et al. Association of extracellular vesicle biomarkers with Alzheimer disease in the baltimore longitudinal study of Aging. *JAMA Neurol*. 2019;76:1340-1351. doi:10.1001/jamaneurol.2019.2462
31. van Deun J, Mestdagh P, Agostinis P, et al.; EV-TRACK Consortium. EV-TRACK: transparent reporting and centralizing knowledge in extracellular vesicle research. *Nature Methods*. 2017;14(3):228-232. doi:10.1038/nmeth.4185
32. Karimi N, Dalirfardouei R, Dias T, Lötvall J, Lässer C. Tetraspanins distinguish separate extracellular vesicle subpopulations in human serum and plasma – Contributions of platelet extracellular vesicles in plasma samples. *J Extracell Vesicles*. 2022;11:e12213. doi:10.1002/jev2.12213
33. Théry C, Witwer KW, Aikawa E, et al. Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the international society for extracellular vesicles and update of the MISEV2014 guidelines. *J Extracell Vesicles*. 2018;7:1535750. doi:10.1080/20013078.2018.1535750
34. Jia L, Qiu Q, Zhang H, et al. Concordance between the assessment of Aβ42, T-tau, and P-T181-tau in peripheral blood neuronal-derived exosomes and cerebrospinal fluid. *Alzheimers Dement*. 2019;15:1071-1080. doi:10.1016/j.jalz.2019.05.002
35. Norman M, Ter-Ovanesyan D, Trieu W, et al. L1CAM is not associated with extracellular vesicles in human cerebrospinal fluid or plasma. *Nat Methods*. 2021;18:631-634. doi:10.1038/s41592-021-01174-8
36. Nogueras-Ortiz CJ, Eren E, Yao P, et al. Single-extracellular vesicle (EV) analyses validate the use of L1 cell adhesion molecule (L1CAM) as a reliable biomarker of neuron-derived EVs. *J Extracell Vesicles*. 2024;13:e12459. doi:10.1002/jev2.12459
37. You Y, Zhang Z, Sultana N, et al. ATP1A3 as a target for isolating neuron-specific extracellular vesicles from human brain and biofluids. *Sci Adv*. 2023;9(37):eadi3647.
38. Hamlett ED, LaRosa A, Mufson EJ, Fortea J, Ledreux A, Granholm AC. Exosome release and cargo in Down syndrome. *Dev Neurobiol*. 2019;79:639-655. doi:10.1002/dneu.22712
39. Mathews PM, Levy E. Exosome production is key to neuronal endosomal pathway integrity in neurodegenerative diseases. *Front Neurosci*. 2019;13:1347. doi:10.3389/fnins.2019.01347
40. Arab T, Mallick ER, Huang Y, et al. Characterization of extracellular vesicles and synthetic nanoparticles with four orthogonal single-particle analysis platforms. *J Extracell Vesicles*. 2021;10:e12079. doi:10.1002/jev2.12079

41. Montoliu-Gaya L, Strydom A, Blennow K, Zetterberg H, Ashton NJ. Blood biomarkers for Alzheimer's disease in Down syndrome. *J Clin Med*. 2021;10:3639. doi:10.3390/jcm10163639
42. Petersen ME, Rafii MS, Zhang F, et al. Plasma total-Tau and neurofilament light chain as diagnostic biomarkers of Alzheimer's disease dementia and mild cognitive impairment in adults with Down syndrome. *J Alzheimers Dis*. 2021;79:671-681. doi:10.3233/JAD-201167
43. Strydom A, Heslegrave A, Startin CM, et al. Neurofilament light as a blood biomarker for neurodegeneration in Down syndrome. *Alzheimers Res Ther*. 2018;10:39. doi:10.1186/s13195-018-0367-x
44. Mielke MM, Dage JL, Frank RD, et al. Performance of plasma phosphorylated tau 181 and 217 in the community. *Nat Med*. 2022;28:1398-1405. doi:10.1038/s41591-022-01822-2
45. Huang YN, Huang JY, Wang CH, Su PH. Long-Term non-congenital cardiac and renal complications in Down syndrome: a study of 32,936 patients. *Children*. 2023;10:1351. doi:10.3390/children10081351
46. Kac PR, Alcolea D, Montoliu-Gaya L, et al. Plasma p-tau212 as a biomarker of sporadic and Down syndrome Alzheimer's disease. *Alzheimers Dement*. 2025;21(4):e70172. doi:10.1002/alz.70172
47. Janelidze S, Christian BT, Price J, et al. Detection of brain Tau pathology in Down syndrome using plasma biomarkers. *JAMA Neurol*. 2022;79:797-807. doi:10.1001/jamaneurol.2022.1740
48. Fagan AM, Henson RL, Li Y, et al. Comparison of CSF biomarkers in Down syndrome and autosomal dominant Alzheimer's disease: a cross-sectional study. *Lancet Neurol*. 2021;20:615-626. doi:10.1016/S1474-4422(21)00139-3
49. Wegiel J, Flory M, Kuchna I, et al. Developmental deficits and staging of dynamics of age associated Alzheimer's disease neurodegeneration and neuronal loss in subjects with Down syndrome. *Acta Neuropathol Commun*. 2022;10:2. doi:10.1186/s40478-021-01300-9
50. D'Acunzo P, Hargash T, Pawlik M, Goulbourne CN, Pérez-González R, Levy E. Enhanced generation of intraluminal vesicles in neuronal late endosomes in the brain of a Down syndrome mouse model with endosomal dysfunction. *Dev Neurobiol*. 2019;79:656-663. doi:10.1002/dneu.22708
51. Boyer E, Deltenre L, Dourte M, et al. Comparison of plasma soluble and extracellular vesicles-associated biomarkers in Alzheimer's disease patients and cognitively normal individuals. *Alzheimer's Res Ther*. 2024;16:141. doi:10.1186/s13195-024-01508-6
52. Winston CN, Goetzl EJ, Akers JC, et al. Prediction of conversion from mild cognitive impairment to dementia with neuronally derived blood exosome protein profile. *Alzheimers Dement*. 2016;3:63-72. doi:10.1016/j.dadm.2016.04.001
53. Oveisgharan S, Arvanitakis Z, Yu L, Farfel J, Schneider JA, Bennett DA. Sex differences in Alzheimer's disease and common neuropathologies of aging. *Acta Neuropathol*. 2018;136:887-900. doi:10.1007/s00401-018-1920-1
54. Schupf N, Pang D, Patel BN, et al. Onset of dementia is associated with age at menopause in women with Down's syndrome. *Ann Neurol*. 2003;54:433-438. doi:10.1002/ana.10677
55. Schupf N, Lee JH, Pang D, et al. Epidemiology of estrogen and dementia in women with Down syndrome. *Free Radic Biol Med*. 2018;114:62-68. doi:10.1016/j.freeradbiomed.2017.08.019
56. Iulita MF, Bejanin A, Vilaplana E, et al. Association of biological sex with clinical outcomes and biomarkers of Alzheimer's disease in adults with Down syndrome. *Brain Commun*. 2023;5:fcad074. doi:10.1093/braincomms/fcad074
57. Startin CM, Ashton NJ, Hamburg S, et al. Plasma biomarkers for amyloid, tau, and cytokines in Down syndrome and sporadic Alzheimer's disease. *Alzheimers Res Ther*. 2019;11:26. doi:10.1186/s13195-019-0477-0

## SUPPORTING INFORMATION

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

**How to cite this article:** Ledreux A, Carmona-Iragui M, Videla L, et al. Cargo of small extracellular vesicles from neuronal origin shows progression of dementia in individuals with Down syndrome. *Alzheimer's Dement*. 2025;21:e70380. https://doi.org/10.1002/alz.70380