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Neuronal exosomes reveal Alzheimer's disease biomarkers in Down syndrome

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Abstract

INTRODUCTION—Individuals with Down syndrome (DS) exhibit Alzheimer's disease (AD) neuropathology and dementia early in life. Blood biomarkers of AD neuropathology would be valuable, as non-AD intellectual disabilities of DS and AD dementia overlap clinically. We hypothesized that elevations of amyloid-beta (A β) peptides and phosphorylated-Tau (P-Tau) in neuronal exosomes may document preclinical AD.

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METHODS—AD neuropathogenic proteins A β ₁₋₄₂, P-T181-Tau and P-S396-Tau were quantified by enzyme-linked immunosorbent assays in extracts of neuronal exosomes purified from blood of individuals with DS and age-matched controls.

RESULTS—Neuronal exosome levels of A β ₁₋₄₂, P-T181-Tau and P-S396-Tau were significantly elevated in individuals with DS compared to age-matched controls at an early age. No significant gender differences were observed.

DISCUSSION—These early increases in A β ₁₋₄₂, P-T181-Tau, and P-S396-Tau in individuals with DS may provide a basis for early intervention as targeted treatments become available.

Keywords

Intellectual disability; Down syndrome; Alzheimer's disease; blood biomarkers; neuronal exosomes; hyper-phosphorylated Tau; amyloid-beta

1. Introduction

Down syndrome (DS) is the most common non-lethal aneuploidy in humans caused by complete or partial trisomy of chromosome 21 [1]. In addition to intellectual disability and atypical development often observed in DS, the prevalence of dementia is significantly higher, with onset at an earlier age than in the general population [2]. A strong candidate mechanism is the amyloid beta precursor protein (APP) gene which is located on chromosome 21 and triplicated in people with DS, giving rise to toxic amyloid peptides at an early age [3]. Amyloid pathology has been observed as early as 15 years of age in postmortem brain samples from individuals with DS [4]. By age 40, amyloid plaques and neurofibrillary tangles are present in sufficient numbers for a postmortem pathological diagnosis of Alzheimer's disease (AD) [5]. Age-related increases in both soluble and insoluble amyloid beta (A β) peptides A β ₁₋₄₀ and A β ₁₋₄₂ within frontal cortex have been described in older individuals with DS, and are correlated with increased oxidative stress, suggesting that other pathological pathways are at play as well [6]. The presence of AD pathology in younger individuals with DS is relatively unknown, due in part to a paucity of brain tissue available for study at earlier age epochs. Biomarkers that reflect AD pathology at earlier ages are thus of considerable interest since neuroprotective therapies will eventually target younger individuals with DS.

Neuropathological biomarkers of AD, including A β ₁₋₄₀ and A β ₁₋₄₂, have been detected in brain tissue and cerebrospinal fluid (CSF) decades prior to onset of dementia in the general population [7], and in DS [8]. Performing lumbar punctures (LPs) in those with DS is challenging; thus there is a need for the development of reliable blood-based biomarkers for AD in this population. Most cell types in the body, including neurons, release small endosomally-derived vesicles, known as exosomes [9, 10]. Exosomes contain proteins, messenger RNA (mRNA) and microRNA (miRNA) that play a prominent role in cellular signaling, removal of unwanted proteins, and transfer of cellular pathogens to other cells [8]. Because of their small size, secreted exosomes diffuse into biological fluids (blood, CSF and urine) and circulate in the interstitial space, both in the brain and the periphery [11].

Neuronal exosomes have unique neuron-specific surface markers, which enable targeted examination from circulating biological fluids [10, 12, 13].

APP processing occurs in exosomes [14–17], and it has been suggested that exosomes play a role in A β clearance [18]. Exosomes receive APP from early endosomes after cleavage into A β peptides, which are secreted from the cells in exosomes [18]. Neuronal exosomes contain A β peptide products and Tau, and transmit these to neighboring cells, other brain regions and the circulatory system, suggesting that neuronal exosomes extracted from either plasma or CSF can specifically assess relevant neuropathological processes within CNS neurons [19, 20]. Other findings indicate that AD biomarkers in neuronal exosomes accurately predict onset of dementia as early as 10 years prior to symptom onset in patients with sporadic AD or frontotemporal dementia (FTD) [10, 21]. Exosomal biomarkers have not been analyzed in individuals with DS. In this study, we hypothesized that neuronal exosomes obtained from blood in DS would have elevated levels of amyloid-beta (A β) peptides and phosphorylated-Tau (P-Tau) that could document a preclinical AD phase in the DS population.

The results presented here are from an international collaboration, including investigators from Hospital de la Santa Creu i Sant Pau in Barcelona (Spain), University of California at Irvine (UCI), Barrow Neurological Institute and Banner Sun Health Research Institute (Arizona), the Linnaeus University in Sweden, and Medical University of South Carolina (MUSC). The aims were to examine alterations in exosome AD biomarkers in children, young adults, and older adults with DS in comparison to age-matched non-DS controls, and also whether AD biomarkers were altered in individuals with DS that display dementia.

2. Methods

2.1. Subject and blood accrual

Blood samples were obtained from 84 individuals (Table 1). Participants either had a normal chromosomal count (Control) or were trisomic for chromosome 21 (DS). The older adult DS group (>35 years) consisted of individuals with DS, independent of dementia status. This group was subdivided into two groups 1) non-cognitively impaired (NCI), and 2) those with early or with fully symptomatic dementia. Because samples were collected from several different clinics, assessed with slightly different cognitive batteries, primary outcomes did not include correlation with clinical measurements in this first study. Blood samples were processed at each clinical site according to standard protocols and aliquots were shipped on dry ice to MUSC. All subjects gave written consent approved by an Institutional Review Board (IRB) or equivalent regulatory agency.

2.2. Diagnosis of cognitive status

The two groups of young controls and young individuals with DS (age 8–35) showed no evidence of cognitive decline by caretaker reports (No Cognitive Impairment; NCI, Table 1). Adult control group (>35 years) were participants in a healthy aging study at the Linnaeus University (Drs. Mohammed and Granholm) or a healthy blood donor study at the UCI Institute for Clinical and Translational Science (Dr. Lott). Age-matched control samples for

the young group (<35 years) were obtained from the National Institutes of Health (NIH). Control donors were free from chronic medical conditions including dementia based on neuropsychological assessments [22, 23].

The participants in the adult DS group (>35 years) were subjected to cognitive testing batteries adjusted to DS criteria (see Supplemental Materials). A consensus diagnosis of non-cognitively impaired (NCI), early symptomatic dementia or fully symptomatic dementia of Alzheimer's-type (DS-AD) was established based on neurological and neuropsychological assessment that included multiple cognitive domains. Dementia was defined by DSM-IV criteria. The term "early symptomatic group" refers to individuals with DS that display only mild signs of dementia, since the term "Mild Cognitive Impairment" (MCI) has not been formally defined for the DS population.

2.3. Isolation of neuronal exosomes

Isolation and analysis of neuronal exosome AD biomarkers was performed by Dr. Edward J. Goetzl using previously described methods, and prior work has demonstrated that the Method employed yields purified samples of exosomes [10] (see Supplemental Materials).

2.4. ELISA analysis of AD biomarkers

Neuronal exosome proteins were quantified using ELISA kits for A β ₁₋₄₂, human P-S396-Tau (Life Technologies/Invitrogen, Camarillo, CA), human P-T181-Tau (Innogenetics Division of Fujirebio US, Inc., Alpharetta, GA), and human CD81 (American Research Products-Cusabio, Waltham, MA), which was used for normalization of biomarkers. The mean value of all CD81 levels for each 96-well plate was set at 1.00 and a normalization value was calculated from the ratio of its CD81 level to that of the mean for the plate. ELISA values for A β ₁₋₄₂, P-T181-Tau and P-S396-Tau were then adjusted proportionally to CD81. Mean levels of one sample set yields levels of that analyte for the same unit mass of exosomes, assuming that the CD81 amount per unit mass of exosomes is the same for all subjects.

We received both serum (n=38) and plasma (n=46) samples, evenly divided between the different groups examined. There was no significant difference in normalized levels of CD81, or in any of the AD biomarkers comparing serum and plasma samples ($p = 0.46$ for A β ₁₋₄₂, $p = 0.084$ for P-T181-Tau, and $p = 0.089$ for P-S396-Tau levels), similar to previous findings from our group [10].

2.5. Statistical analyses

2.5.1. Population tests—We first examined differences in AD markers between the control non-DS group and cases with DS. Grubb's method was used to check for outliers in each neuronal exosome biomarker which resulted in 2 out of 84 values excluded from the A β ₁₋₄₂ and P-S396-Tau data sets. D'Agostino-Pearson normality tests were performed on control non-DS and DS groups for each neuronal exosome biomarker to test suitability for parametric analyses. Linear regression analyses were performed for each neuronal exosome biomarker over age to assess potential age effects. Pearson correlation analyses were

performed to determine whether age affected group differences in terms of the neuronal exosome AD biomarkers.

2.5.2. Statistical tests—Since the incidence of AD is known to be higher in individuals with DS after the age of 35 [1], the total population was subdivided at the age of 35 in order to determine differences between age (younger: < 35 years, older: >35 years), DS (control-DS) and interaction effects. A two-way ANOVA examined the interaction between dependent variables, age x DS, for each biomarker, followed by Tukey's post hoc test for multiple comparisons. Student's t tests were used to test for gender differences within each group for each neuronal exosome biomarker. The older adult DS group (>35 years) consisted of all individuals with DS, independent of dementia status. This group was subdivided into two groups 1) non-cognitively impaired (NCI), and 2) those with early or with fully symptomatic dementia. Student's t tests were used to test for significant differences between these groups for each neuronal exosome biomarker. Statistical analyses were performed with GraphPad Prism 6 (GraphPad Software, Inc., La Jolla, CA) with alpha set at 0.05.

3. Results

3.1. Participant demographics

Participant demographics are shown in Table 1. The term non-cognitively impaired (NCI) signifies individuals who were cognitively stable at the time of blood donation. The mean age of non-DS controls and individuals with DS for each age group was not significantly different.

3.2. Effects of DS and age on AD biomarkers

$A\beta_{1-42}$, P-T181-Tau and P-S396-Tau were significantly elevated in the DS population relative to the control population (Fig. 1A–C; Student's t-test, $p < 0.0001$ for each biomarker; $n = 37$ for control non-DS, and $n = 47$ for DS participants).

We further divided the control and DS groups into two age categories, below 35 and above 35 years old and performed a two-way ANOVA to test for interaction. For all three neuronal exosome biomarkers, there was a highly significant effect of DS, with no age effect and no significant interaction between age and trisomy status. For $A\beta_{1-42}$ levels (Fig. 2A; $F_{1,78} = 44.46$, $p < 0.0001$), Tukey's post hoc analyses revealed significant elevations in young DS ($p = 0.024$) and older DS groups ($p < 0.0001$) compared to age-matched controls. For P-T181-Tau levels (Fig. 2B; $F_{1,80} = 64.53$, $p < 0.0001$), Tukey's post hoc analyses revealed significant elevations in young and older DS groups (both $p < 0.0001$) compared to age-matched controls. For P-S396-Tau levels (Fig. 2C; $F_{1,78} = 50.76$, $p < 0.0001$), Tukey's post hoc analyses revealed significant elevations in both young ($p = 0.002$) and older DS groups ($p < 0.0001$), compared to age-matched controls. The values for young and old subgroups for controls and subjects with DS were not significantly different for any biomarker tested. These findings demonstrated that AD biomarkers were significantly elevated in those with DS compared to age-matched controls.

A linear regression analysis across age was performed to determine whether there were significant aging effects (Figure 3). $A\beta_{1-42}$ levels were 210% greater (Fig. 3A; $F_{1,79} = 67.71$,

$p < 0.0001$) in DS (mean: 6.74 ± 1.71) compared to control (mean: 2.17 ± 0.61) group. However, there was no association between age and $A\beta_{1-42}$ levels for the non-DS control group ($p = 0.729$) or the DS group ($p = 0.454$), indicating no significant age effects on $A\beta_{1-42}$ levels, even when all DS groups were included in the analysis. We observed a significant doubling of P-T181-Tau levels (Fig. 3B; $F_{1,81} = 98.16$, $p < 0.0001$) for DS (mean: 146.3 ± 27.73) relative to the non-DS control group (mean: 71.92 ± 13.40). The slope of P-T181-Tau levels was also not significantly different from zero for either group: non-DS control ($p = 0.861$) and DS ($p = 0.076$). Similarly, the levels of P-S396-Tau were 76% greater (Fig. 3C; $F_{1,79} = 69.61$, $p < 0.0001$) for the DS group (mean: 22.87 ± 4.09) relative to the non-DS control group (mean: 12.95 ± 1.68). The slope of P-S396-Tau levels was not significantly different from zero for either group: non-DS control ($p = 0.069$) and DS ($p = 0.962$). Levels of all biomarkers were elevated in the age stratified DS groups compared with the age-matched controls, suggesting at least a 2-fold elevation for participants with DS.

In addition to linear regression, we tested for correlation of biomarkers to each other and age in each group. Pearson correlation analyses between neuronal exosome biomarkers revealed that $A\beta_{1-42}$ levels were significantly correlated with P-S396-Tau ($r = 0.438$, $p = 0.008$) and a trend for P-T181-Tau and P-S396-Tau ($r = 0.316$, $p = 0.059$) in the non-DS control group. $A\beta_{1-42}$ significantly correlated with P-T181-Tau ($r = 0.338$, $p = 0.022$) and P-T181-Tau significantly correlated with P-S396-Tau ($r = 0.32$, $p = 0.029$) in the DS group. Although P-T181-Tau was significantly elevated at younger ages, it also showed a trend towards further elevation with age in the DS group ($r = 0.261$, $p = 0.076$).

3.3. Gender effects on AD biomarkers

Student's *t* tests revealed no significant difference ($p > 0.10$) between male and female participants for the $A\beta_{1-42}$ or the P-S396-Tau levels, but showed a significant gender effect for the P-T181-Tau levels only in the older controls ($p = 0.05$) and the older DS group ($p = 0.02$) (Table 2). Since there was no gender effect for P-T181-Tau levels for any young groups, we excluded gender from further analyses and focused on dementia-related effects within the DS participants.

3.4. Effects of dementia status in aged DS subjects

To investigate the effect of dementia status on the exosome biomarkers levels, we further divided the adult DS group into two categories: 1) those with NCI and 2) those with early symptomatic dementia or with fully symptomatic dementia indicative of DS-AD. Because there is controversy regarding MCI diagnosis in those with DS, we chose to collapse these two groups. $A\beta_{1-42}$ levels were significantly decreased (Student's *t* test, $p = 0.048$) in adults with DS and dementia symptoms, relative to those with no apparent dementia (Figure 4A). P-T181-Tau levels were not significantly different between the two groups (Figure 4B). By contrast, P-S396-Tau levels were significantly elevated (Student's *t*-test, $p = 0.008$) in participants with early or fully symptomatic dementia compared to those with DS and no dementia (Figure 4C). Taken together, these data suggest that neuronal exosome levels of $A\beta_{1-42}$, and P-Tau follow different trajectories in adults with DS who exhibit symptoms of dementia.

4. Discussion

We report A β_{1-42} and P-Tau levels from neuronal exosomes isolated from individuals with DS, as a function of age and dementia status. We found a marked increase in AD biomarkers in individuals with DS as early as 8 years of age. Extrapolation of the linear regressions suggests that these abnormal neuronal exosome biomarker levels may be elevated already at birth in DS, but further studies are needed. Adults with DS diagnosed with dementia exhibited lower A β_{1-42} and higher P-S396-Tau levels compared to non-cognitively impaired adults with DS, suggesting different biomarker trajectories in exosomes as a consequence of the AD process.

It is not surprising that A β_{1-42} levels were elevated early in DS because the APP gene is located on chromosome 21 [24,25], and elevated toxic amyloid peptides in the plasma of young adults with DS have been reported [26]. A positron emission tomography (PET) imaging study, using the beta-amyloid Pittsburgh compound-B (PIB), showed increased load of fibrillar A β in DS, in those >35 years of age [27]. In contrast, others have shown lower A β_{1-42} levels in plasma in individuals with DS diagnosed with dementia compared to subjects with DS but no dementia [28]. Diffuse amyloid plaques have been described as early as age 15 in brain samples from people with DS [29, 30]. Increased levels of A β_{1-42} have also been reported in the CSF of adults [1,31,32] and children [8] with DS. The Dominantly Inherited Alzheimer Network (DIAN) showed reduced concentrations of CSF A β_{1-42} and elevated concentrations of CSF P-Tau, in asymptomatic mutation carriers 10 to 20 years before their estimated age at symptom onset [32], data that may lend support to the current findings [33]. The sensitivity discrepancy between PET imaging versus plasma and CSF studies may reflect PIB binding affinity preferences to protein-lipid components [34] or PIB tracer signal strength. Longitudinal studies need to be performed in the DS population to address potential differences in sensitivity of different diagnostic methods.

Since the Tau gene is not known to be affected by trisomy 21, it is more surprising that P-Tau levels are elevated early in DS. Phosphorylation of Tau protein at the carboxyl terminus has been shown to be one of the earliest pathological events in both AD and in DS-AD [32], and may occur prior to accumulation of brain fibrillary P-Tau. Here, we found that neuronal exosome levels of both P-T181-Tau and P-S396-Tau were significantly increased in younger individuals with DS compared to age-matched controls and that hyper-phosphorylation persisted into late adulthood when DS co-occurred with AD dementia. The highest levels of exosomal P-S396-Tau were found in cases diagnosed with dementia (Fig. 4C), suggesting that P-Tau continues to accumulate in exosomes as AD pathology progresses. Our data support previous P-Tau observations regarding AD-related progression in adults with DS but also suggest that significant elevations occur, at least in neuronal exosomes, as early as 8 years of age in DS. Future studies targeting earlier age ranges in DS should be undertaken to gain insight into the early neuropathological processes associated with DS. The data reported here may also bolster new *in vivo* imaging studies, using novel P-Tau radioligands [35]. The biological mechanisms driving the elevated P-Tau levels early in those with DS remain unknown.

Potential mechanisms for Tau dysregulation can be found in several genes involved in the trisomy. For example, superoxide dismutase 1 (SOD1) is located on the triplicated segment of Chr. 21, and is involved in oxidative stress which exacerbates AD pathology in the DS brain [36]. Dysregulation of alternative splicing of Tau has been reported in DS as well as in AD [37] and may also underlie the abnormal hyper-phosphorylation of Tau [38]. Furthermore, two genes located on Chromosome 21, the dual-specificity tyrosine phosphorylation-regulated kinase 1A (DYRK1A) gene, and regulator of calcineurin 1 (RCAN1), are highly expressed in the DS brain [39–41], and have been implicated in the dysregulation of Tau phosphorylation associated with early onset AD in DS [42, 43, 44]. Taken together, dysregulation of these genes suggest several alternative molecular mechanisms underlying the increase in A β ₁₋₄₂, P-S396-Tau and P-T181-Tau observed in neuronal exosomes isolated from children with DS, which will be further explored in ongoing expanded studies.

The capacity to quantify A β ₁₋₄₂ and P-Tau in neuronal exosomes from easily acquirable blood samples has permitted us to show significant abnormalities in these proteins at an early age in individuals with DS. As expected, due to the increase in APP gene dosage, A β ₁₋₄₂ levels were increased throughout the lifespan in participants with DS, but paradoxically were significantly reduced in those with a clinical diagnosis of dementia. The decrease in neuronal exosome A β ₁₋₄₂ levels paralleled A β ₁₋₄₂ decreases in plasma observed with conversion to AD in the DS population [28]. If exosomes express intra-neuronal signatures that are unique compared to the extracellular environment reflected by CSF or serum biomarkers, one could hypothesize that neuronal exosome A β ₁₋₄₂ has a distinct interface with extracellular plaques in the brain compared to serum and CSF A β ₁₋₄₂. Measurements of serum and CSF A β ₁₋₄₂ occurring concurrently with A β plaque formation and cognitive impairment in those with DS-AD [25, 26, 28] may give different and complementary results compared to neuronal exosome A β ₁₋₄₂ and longitudinal studies will have to be performed to test this hypothesis. Also, future studies will be needed to clarify important questions about both inter- and intra-exosomal amyloid peptide production, effects on different populations of amyloid deposits and correlations with clinical dementia status in the DS population. While amyloid-beta is a central concern, elevated Tau signatures are paramount as well. P-S396-Tau and P-T181-Tau levels were increased early in life, and P-S396-Tau showed a significant further increase in dementia suggesting that Tau phosphorylation plays a role in dementia onset and progression in DS.

We suggest that the neuronal exosome approach offers several benefits for the interrogation of early events in AD pathogenesis. Another significant benefit is that outcome measures can be reliably obtained either from serum or plasma samples, since data are normalized against the same neuronal marker. Finally, a neuronal exosome approach may prove useful in evaluating potential drug targets for early intervention of AD, while enabling reliable measures of therapeutic efficacy. Future studies will include comparison studies between whole plasma and neuronal exosome amyloid levels, correlation studies between exosome markers and cognitive measures, and an expanded search for other biomarkers in neuronal exosomes that may predict dementia and/or other conditions in adults with DS.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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A. Overall neuronal exosome $A\beta_{1-42}$ levels **B. Overall neuronal exosome P-T181-Tau levels** **C. Overall neuronal exosome P-S396-Tau levels**

Figure 1. Overall neuronal exosome AD biomarkers isolated from blood of control and DS populations

1A. $A\beta_{1-42}$: participants with DS exhibited significantly elevated levels ($p < 0.0001$) compared to age-matched control participants. **1B.** P-T181-Tau: participants with DS exhibited significantly elevated levels ($p < 0.0001$) compared to age-matched controls. **1C.** P-S396-Tau: participants with DS exhibited significantly elevated levels ($p < 0.0001$) compared to age-matched controls. Error bars represent $\pm$ SEM. $N = 37$ for non-DS control participants, and $N = 47$ for participants with DS (see Table 1).

A. Neuronal exosome $A\beta_{1-42}$ levels **B. Neuronal exosome P-T181-Tau levels** **C. Neuronal exosome P-S396-Tau levels**

Figure 2. Group differences in AD biomarkers in neuronal exosomes isolated from blood of individuals with and without DS of different ages

Age was divided into those below 35 years of age vs. those above 35 years of age. Note that both young and older individuals with DS exhibited significantly increased levels of all three AD biomarkers, compared to age-matched controls. **2A.** $A\beta_{1-42}$: younger participants with DS exhibited significantly elevated levels ($p = 0.024$) compared to age-matched controls. Older participants with DS exhibited significantly elevated levels compared to age-matched controls ($p < 0.0001$). **2B.** P-T181-Tau: younger participants with DS exhibited significantly

elevated levels ($p = 0.001$) compared to age-matched controls. Older participants with DS exhibited significantly elevated levels compared to age-matched controls ($p < 0.0001$). **2C.** P-S396-Tau: younger participants with DS exhibited significantly elevated levels ($p = 0.002$) compared to age-matched controls. Older participants with DS exhibited significantly elevated levels compared to age-matched controls ($p < 0.0001$). Error bars represent $\pm$ SEM.

A. Neuronal exosome $A\beta_{1-42}$ levels by age **B. Neuronal exosome P-T181-Tau levels by age** **C. Neuronal exosome P-S396-Tau levels by age**

Figure 3. Effects of age on AD biomarkers in neuronal exosomes isolated from blood samples of individuals with and without DS

No age effects were observed for either $A\beta_{1-42}$, P-T181-Tau or P-S396-Tau. **3A.** $A\beta_{1-42}$: There was no significant effect of age on $A\beta_{1-42}$ levels in the DS group. The y-intercepts for DS group and non-DS control group were significantly different ($p < 0.0001$). Age-matched control participants did not exhibit any main effects of age. **3B-C.** P-T181-Tau and P-S396-Tau: No age effects were observed for either P-T181-Tau or P-S396-Tau. However, the y-intercepts for both P-T181-Tau and P-S396-Tau were significantly different ($p < 0.0001$)

between the DS group and age-matched control group. Dotted lines represent the 95% confidence band of the best fit line.

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A. Neuronal exosome $A\beta_{1-42}$ levels by clinical diagnosis

B. Neuronal exosome P-T181-Tau levels by clinical diagnosis

C. Neuronal exosome P-S396-Tau levels by clinical diagnosis

Figure 4. Neuronal exosome levels of AD biomarkers by clinical diagnosis

4A. $A\beta_{1-42}$: Student's t test revealed a significant decrease in $A\beta_{1-42}$ levels for adults with early symptomatic or fully symptomatic dementia compared to no apparent dementia subgroup ($p = 0.048$). **4B.** P-T181-Tau: There was no significant overall effect between the two groups. **4C.** PS396-Tau: Student's t test revealed a significant increase for DS adults diagnosed with early or fully symptomatic dementia relative to DS adults with no apparent dementia. Error bars represent $\pm$ SEM. Error bars represent $\pm$ SEM.

Table 1

Participant demographics

Groups	Diagnosis	n =	Male/Female	Average age (yr) ± SEM	Age range (yr)
Young controls (8–35)	NCI	9	4/5	15 ± 2.5	8–27
Adult controls (>35)	NCI	28	13/15	55 ± 1.7	43–77
Young DS (8–35)	NCI	14	8/6	19 ± 2.2	8–31
Adult DS (>35)	NCI Early or fully symptomatic dementia	33	18/15	51 ± 0.8	43–62
Subgroups:		16	10/6	51 ± 1.1	43–60
		17	8/9	51 ± 1.4	43–62

Table 2

Male and female average neuronal exosome biomarker levels ($\pm$ SEM).

	A β ₁₋₄₂			P-T181-Tau			P-S396-Tau		
	Female	Male	P value	Female	Male	P value	Female	Male	P value
Control (<35)	2.97 $\pm$ 0.55	2.41 $\pm$ 0.59	0.51	68.0 $\pm$ 14.88	96.6 $\pm$ 25.30	0.34	11.3 $\pm$ 1.72	14.0 $\pm$ 2.47	0.38
DS (<35)	7.05 $\pm$ 0.91	6.50 $\pm$ 1.17	0.73	194.3 $\pm$ 30.23	159.5 $\pm$ 16.56	0.30	27.7 $\pm$ 2.97	20.9 $\pm$ 2.85	0.13
Control (>35)	2.17 $\pm$ 0.48	1.25 $\pm$ 0.20	0.10	81.7 $\pm$ 7.18	60.8 $\pm$ 6.89	0.05	9.47 $\pm$ 0.92	9.02 $\pm$ 1.19	0.77
DS (>35)	8.61 $\pm$ 1.12	8.37 $\pm$ 1.16	0.88	169.6 $\pm$ 17.34	227.9 $\pm$ 15.68	0.02	23.8 $\pm$ 2.54	22.5 $\pm$ 2.01	0.71

Controls: n= 17 males; n=20 females, DS: n=26 males; n=21 females.