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## Synaptophysin and synaptojanin-1 in Down syndrome are differentially affected by Alzheimer disease

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### Abstract

Adults with Down syndrome (DS) develop Alzheimer's disease (AD) neuropathology by 40 years of age. Synaptophysin (SYN) consistently declines with age and is further reduced with sporadic AD. Thus, we hypothesized that SYN would be reduced in DS with AD. The gene for synaptojanin-1 (SYNJ1), involved in synaptic vesicle recycling, is on chromosome 21. We measured SYN and SYNJ1 in an autopsy series of 39 cases with DS and 28 without DS, along with 7 sporadic AD cases were examined. SYN was significantly lower in DSAD compared with DS alone and similar to sporadic AD. Reduced SYN is associated with AD neuropathology and with A $\beta$  levels in DS, as is seen in sporadic AD. SYNJ1 was significantly higher in DS and correlated with several measures of A $\beta$ . SYNJ1 was higher in DSAD and significantly higher than SYNJ1 in sporadic AD. Although significantly higher in DS, SYNJ1 is further increased with AD neuropathology suggesting interesting differences in a synapse-associated protein that is overexpressed in trisomy 21.

### Keywords

beta-amyloid; neuroinflammation; oligomers; synapses; synaptophysin; synaptojanin; trisomy 21

## Introduction

The most common known genetic cause of intellectual disability is Down syndrome (DS), also called trisomy 21. The primary cause of DS is triplication of chromosome 21 [1], resulting in a phenotype that is accompanied by altered brain development and other neurologic features [2,3]. The lifespan of adults with DS has been improving, leading to a higher prevalence of individuals with DS at middle and older ages. [4]. However, a key challenge for adults with DS as they age is the increasing risk for developing Alzheimer's disease (AD) [5]. Sporadic AD is a progressive neurodegenerative disease associated with cognitive decline and dementia, the pathological hallmarks of which are beta-amyloid (A $\beta$ ) plaques and neurofibrillary tangles (NFTs) in the brain. AD neuropathology (including A $\beta$  plaques/deposition and NFTs) appears in virtually all adults with trisomy 21 after 40 years of age [6-8]. A $\beta$  is cleaved from the larger beta-amyloid precursor protein which is encoded on chromosome 21, and likely accounts for increased amount of A $\beta$  deposition seen in DS. [9].

A consistent observation in the brains of individuals with sporadic AD in comparison to brain specimens from nondemented elderly control brains is synapse loss, a finding which reflects dementia severity [10-12]. In addition, several studies show a reduction in proteins associated with synapses, with one of the most robust observations being a reduction in synaptophysin (SYN)[13]. SYN is a 38 kDa integral membrane glycoprotein located in presynaptic vesicles [14]. Previous studies in individuals with AD report SYN protein losses (e.g. [15-18]). There are a few papers describing SYN loss in autopsy samples from older adults with DS [19,20], but the sample sizes are small and none were characterized as having AD. There is a need for a more systematic evaluation of the age of onset of SYN protein losses relative to AD neuropathology in DS.

Another synapse associated protein that is of key interest in DS is synaptojanin 1 (SYNJ1). The gene for SYNJ1 is on chromosome 21 and present in triplicate in DS [21]. SYNJ1 is a brain enriched phosphoinositide phosphatase [22] that is involved with endocytosis and synaptic vesicle cycling [23,24]. Several studies report increased protein levels of SYNJ1 in DS brain [25], particularly within the frontal cortex [26] using immunocytochemical or western blotting approaches. Interestingly, increasing SYNJ1 can ameliorate synaptic and behavioral impairments in a mouse model of AD, Tg2576 mice [27]. SYNJ1 expression declines in the brain of individuals with the sporadic form of AD [28] but this finding has not been tested in DS. However, there are no reports of the effects of aging or the presence of AD in DS with respect to SYNJ1 levels.

This study examined the hypothesis that SYNJ1 would be higher overall in DS due to overexpression but may also decline in DS with AD (DSAD). We also hypothesized that SYN would be reduced in DSAD as reported in sporadic AD. Further, we predicted that SYN and SYNJ1 protein levels would be decreased with increasing AD neuropathology as characterized in part by, A $\beta$  and oligomeric A $\beta$  accumulation. To test these hypotheses, we conducted western blotting studies of SYN and SYNJ1 protein in frontal cortex samples from DS and DSAD autopsy cases in comparison to age-matched control cases and sporadic AD cases. Previously published A $\beta$  measures combined with the western blot measures of

SYN and SYNJ1 from the current study allowed us to look at changes in synaptic protein levels with A $\beta$  accumulation.

## Materials and Methods

### Subjects

Autopsy brain tissue was obtained from several sources including the University of California at Irvine Alzheimer's Disease Research Center and the Maryland Developmental Disorders Brain Bank. Human tissue collection and handling conformed to University of Kentucky/UC Irvine Institutional Review Board guidelines. All cases selected ranged from 1 to 88 years based, in part, on the availability of frozen frontal cortex. Control cases were subsequently selected to match for age and PMI as closely as possible to the DS cases. AD cases were selected based on PMI only to match DSAD cases (DS cases that had sufficient neuropathology for a post-mortem diagnosis of AD)[29]. Since individuals with DSAD typically come to autopsy at younger ages than sporadic AD cases, it was not possible to match for age at death. As a result, tissues from 5 autopsy groups were available: young controls (YC, age matched to young DS; N=13), old controls (OC, age matched to DSAD; N=15), DS (N=8), DSAD (N=31), and AD alone (N=7). Both males and females were included in the study, but given the challenges of matching cases, we did not match for gender. The level of premorbid ID was not available in these cases and thus it was not possible to use this variable as a co-variate in the analysis.

As shown in Table 1, DS cases were divided into two groups: with findings for AD (DSAD) or without (DS). In the former group, we required sufficient pathology for a diagnosis of AD [30]. All DSAD cases were over the age of 40 years. Thus for the current study, control cases were split into two groups, less than or equal to 40 years (YC) or older than 40 years (OC) at death. The post mortem interval (PMI) was different across groups, with the DSAD group overall having a lower PMI ( $F(4,73)=7.3$   $p<.0005$ ). Group comparisons of SYN and SYNJ1 were thus adjusted for PMI when necessary.

### Western blotting

Frozen samples from the mid-frontal cortex (BA46) were homogenized in 1% SDS extraction buffer (150 mg/ml) with protease inhibitors (Roche, Indianapolis, IN). Protein concentration was determined by BCA as described previously [31]. A 10-20% SDS page criterion gel (Bio-Rad Laboratories, Hercules, CA) was used to separate proteins and transferred to a nitrocellulose membrane (Bio-Rad Laboratories). Membranes were probed with anti-synaptophysin (Millipore Corp., Temcula, CA, 1:800,000, Monoclonal) or anti-synaptojanin 1 (Sigma Prestige Antibodies, St. Louis, MO, 1:1000, Polyclonal) and then incubated with either anti-rabbit or anti-mouse secondary antibody (Santa Cruz Biotechnology, Santa Cruz, CA). Detection was done using Super Signal West Pico Chemiluminescent Substrate (Thermo Scientific, Rockford, IL). To ensure that densitometric measurements of protein levels of SYN and SYNJ1 were not saturated, a standard curve was derived for each antibody as published in a previous study [31]. To establish equal protein loading, membranes were stripped reprobed with anti- $\alpha$ -tubulin (Abcam, Cambridge, MA, 1:80,000, Monoclonal)

## A $\beta$ ELISAs

A $\beta$  was extracted from tissue measured as previously described [32]. Briefly, frozen cortical samples were extracted sequentially in ice cold phosphate buffered saline (PBS, pH 7.4) with a complete protease inhibitor cocktail (PIC; Amresco, Solon, OH) and centrifuged at  $20,800 \times g$  for 30 min. at 4°C. Following centrifugation, the supernatant was collected and the pellets were sonicated (10  $\times$  0.5 sec pulses at 100W, Sonic Dismembrator, Fisher Scientific, Pittsburgh, PA) in 2% SDS with PIC and centrifuged at  $20,800 \times g$  for 30 min. at 14°C. The supernatant was again collected and the remaining pellets were sonicated in 70% formic acid (FA), followed by centrifugation at  $20,800 \times g$  for 1 hour at 4°C. Samples were stored at  $^{\circ}80^{\circ}\text{C}$  until time of assay.

FA-extracted material was initially neutralized by a 1:20 dilution in TP buffer (1 M Tris base, 0.5 M Na<sub>2</sub>HPO<sub>4</sub>), followed by a further dilution as needed (1:5 to 1:20, for a final dilution of 1:100 to 1:400) in Antigen Capture buffer (AC) (20mM Na<sub>3</sub>PO<sub>4</sub>, 0.4% Block Ace (AbD Serotec, Raleigh, NC), 0.05% NaN<sub>3</sub>, 2mM EDTA, 0.4M NaCl, 0.2% BSA, 0.05% CHAPS, pH 7) . SDS soluble fractions were diluted as needed (1:20 to 1:50) in AC buffer alone. PBS fractions were diluted 1:4 in AC buffer alone.

A $\beta$  was measured using a standard, well-characterized two-site sandwich ELISA as described previously [47]. Briefly, an Immulon 4HBX plate was coated with 0.5  $\mu\text{g}$  antibody per well, incubated overnight at 4°C, and blocked with a solution of Synblock (AbD Serotec, as per the manufacturer's instructions). Antigen capture was performed using monoclonal antibody Ab9 (against Human A $\beta$  1-16). Antigen detection was performed using biotinylated antibodies 13.1.1 (end-specific for A $\beta$  1-40) and 12F4 (end-specific for A $\beta$  1-42; Covance, Princeton, NJ), followed by NeutraVidin-HRP (Pierce Biotechnologies, Rockford, IL)

A synthetic A $\beta$  peptide standard was run on the same plate for comparison, and standards and samples were run at least in duplicate; A $\beta$  values were determined by interpolation relative to the standard curve. Plates were washed between steps with standard PBS containing 0.05% Tween-20 (2-4x) followed by PBS (2-4x). Plates were developed with TMB reagent (KPL, Inc., Gaithersburg, MD), stopped with 6% *o*-phosphoric acid, and read at 450 nm using a multiwell plate reader (BioTek, Winooski, VT).

Oligomeric A $\beta$  from the SDS-soluble fraction was measured using a single-site sandwich ELISA similar to the one described above, except the same antibody (4G8; Covance, Princeton, NJ) was used for capture and detection. SDS samples were diluted 1:50 in AC buffer. Synthetic A $\beta$ 42 oligomers were used to prepare a standard curve; oligomeric A $\beta$  values were determined by interpolation relative to the standard curve.

## Data Analysis

For synapse protein measures, multiple western blots were required. In order to standardize across gels, one DS with AD case was loaded on each gel with increasing concentrations (1,5,10  $\mu\text{g}$ ) to serve as an internal control [31] . Optical density measures for each gel were then calculated as total protein ( $\mu\text{g}$ ) of this internal control case. We examined the relationship between synapse markers and post mortem interval to determine if this needed

to be included as a covariate in subsequent analyses. In each group analysis, cases were classified as YC (<40 yrs), DS (<40), OC (>40 years), DSAD (>40) and AD. The AD, DS and DSAD groups were compared to the appropriate control group (YC, OC). Using this method, we were able to determine whether protein expression differed from the control group. A two-way univariate analysis of variance was used to compare group differences (Young <40 & >40 Old; AD present vs. not present) with gender serving as a co-variate. Post-hoc pairwise comparisons of the means for all analyses were made using the Bonferroni test. Pearson correlation coefficients were used to test for the linear association between synapse proteins and age within the DS and control groups, separately. To determine the correlation between SYN and SYNJ1 with measures of A $\beta$ , we first log transformed the raw A $\beta$  data ( $\log_{10}+10$ ) as most associations were nonlinear and calculated partial correlations with age as a co-variate. We further confirmed correlations between SYN and SYNJ1 with A $\beta$  using a stepwise linear regression that included age at death, gender and PMI.

## Results

Figure 1 shows a representative western blot experiment, a single band representing SYN was observed at ~38 kDa and SYNJ1 showed 1 or 2 bands at 140 and 180 kDa (Fig 1A). Given differences in PMI across our groups, we calculated the correlation between PMI and our outcome measures. Longer PMI was associated with higher protein levels of SYN ( $r=0.40$   $p<.0005$ ) but not with SYNJ1. Therefore, we decided to adjust for PMI in analyses involving SYN.

As a first level of analysis, a comparison of the five groups (YC, OC, DS, DSAD and AD) was made using an analysis of variance and the Bonferroni correction was used for *post hoc* comparisons. Significant group differences were found for SYN ( $F(4,73)=2.75$   $p=0.035$ ) and for SYNJ1 ( $F(4,73)=3.2$   $p=0.017$ ). Figure 1B shows that SYN was significantly lower in DSAD and AD cases compared to DS or both YC and OC. SYNJ1 levels were lowest in the AD cases relative to younger controls and to DS cases and significantly lower than DS with AD cases Fig. 1C).

Next, we used a two factor univariate analysis that compared genotype (DS vs. CTL) and group (<40 or >40 years for controls vs. DS or DSAD) to determine the contribution of each factor independently to SYN and SYNJ1 protein levels (Table 2). Sporadic AD cases were not included in this analysis. For SYN, after co-varying for PMI, there was a significant main effect of genotype (i.e. the presence of DS) ( $F(1,67)=3.36$   $p=0.07$ ). If PMI is removed from this analysis to increase the power, the presence of DS is a significant contributor to SYN protein levels ( $F(1,67)=3.91$   $p=0.05$ ) as is the age group (<40 years vs > 40 years) ( $F(1,67)=6.4$   $p=0.014$ ). The interaction was not significant suggesting that with age in controls and DSAD, there is a parallel decrease in SYN protein level. Overall, in DSAD SYN protein levels were ~50% of SYN levels in DS alone.

SYNJ1 was significantly different overall in DS when compared to control cases ( $F(1,67)=5.1$   $p=0.03$ )(Table 2). SYNJ1 also differed by age group (Young vs. Old) in controls and DSAD cases ( $F(1,67)=6.4$   $p=0.014$ ). The interaction between age group and

presence of DS was not significant ( $F(1,67)=0.16$   $p=0.69$ ). The lack of interaction effect may be due to opposite effects of age or the presence of AD neuropathology in controls and DS cases, respectively. In controls, there was a lowering of SYNJ1 levels in cases over 40 years (young mean=5.70 ( $\pm 2.57$ ); old mean=4.33( $\pm 3.03$ ); there was an opposite effect with increasing levels of SYNJ1 observed in DSAD (DS mean=7.77 ( $\pm 7.51$ ); DSAD mean=10.97 ( $\pm 10.73$ ). In DS overall, SYNJ1 levels were ~25% higher than age-matched controls. SYNJ1 levels were increased ~90% in DSAD cases as compared to OCs, and ~40% increased as compared to DS without AD.

We next determined whether synapse protein levels declined as a function of age in controls or DS groups, separately. Within the control group only (no sporadic AD cases), there were no significant age-associated decreases in SYN or SYNJ1. If sporadic AD cases were added into the analysis – there was a significant correlation between age at death and SYN ( $r=-0.45$   $p=0.007$ ) and SYNJ1 ( $r=-0.35$   $p=0.039$ )(Fig 2A and C). In DS cases, SYN ( $r=-0.48$   $p=0.002$ ) decreased as a function of age (Fig.2B) but no significant age effects were observed for SYNJ1 (Fig. 2D).

Increasing A $\beta$  and A $\beta$  oligomer accumulation is associated with the disruption of synaptic function (reviewed in [33-35]). Thus, we compared A $\beta$  and A $\beta$  oligomer levels to the amount of SYN and SYNJ1 in DS only using partial correlations that corrected for age at death given that this comparison includes both age as well as AD neuropathology and it is challenging to distinguish these two variables. Higher amounts of oligomeric A $\beta$ , SDS-soluble A $\beta$  1-40 and SDS-soluble A $\beta$  1-42 were associated with lower SYN protein levels (Table 3). Exploratory stepwise regressions predicting SYN and SYNJ1 from all of the A $\beta$  measures (including PMI, genotype and age at death) confirmed the association between SYN level and SDS-soluble A $\beta$  1-42 ( $R^2=0.16$ ,  $p=0.001$ ). For SYNJ1, the best predictor was FA extracted A $\beta$  1-42 ( $R^2=0.074$   $p=0.03$ ) but this was much weaker than that seen for SYN with SDS A $\beta$  1-42).

## Discussion

SYN protein levels in the frontal cortex of DSAD were significantly lower than DS alone and comparable to AD in persons without DS. Further, lower SYN was associated with higher soluble (SDS) A $\beta$  and oligomeric A $\beta$  levels in DS cases. SYN was also correlated with the amount of oligomeric and SDS extracted A $\beta$  in cases with DS. These results suggest that as with sporadic AD, SYN is significantly reduced in DSAD and is associated with A $\beta$  neuropathology. In contrast, higher overall levels of SYNJ1 were observed in DS, which is consistent with overexpression. However, novel results in the current study suggest that in contrast to our original hypothesis that SYNJ1 would decrease with DSAD, we found a further increase in SYNJ1 in DSAD.

Previous studies of AD robustly report synaptic protein losses and particularly in SYN (e.g. [15-18]). These previous findings formed the rationale for selecting SYN as a protein marker to determine if there was a similar synaptic protein loss in DSAD compared to sporadic AD. The presence of AD neuropathology in DS was also associated with a similar level of SYN as in sporadic AD, which in turn was significantly lower than in DS cases

without AD, suggesting this protein is affected by AD overall. In sporadic AD, SYN is associated with cognition and lower SYN is associated with poorer cognition [17]. Interestingly, higher SYN is observed in autopsy cases with normal cognition but with significant AD neuropathology at autopsy when compared to typical AD cases suggesting a possible protective effect or that SYN is critical to intact cognition [31,36]. Similar studies have not been described in DS primarily due to the challenge of obtaining cognitively characterized autopsy cases, but it is likely that a similar link is present.

SYNJ1 is a brain-enriched phosphoinositide phosphatase [22] that is engaged during endocytosis and synaptic vesicle cycling [23]. There is also evidence that SYNJ1 is involved with astrogliosis [37] and overexpression of SYNJ1 by 50% in Ts65Dn mice is associated with a 36% increase in glial fibrillary acidic protein (GFAP) but not S100 $\beta$  (a gene also on Chromosome 21) levels. SYNJ1 protein levels are increased in the DS brain [25], particularly within the frontal cortex [26]. SYNJ1 is overexpressed in the Ts65Dn mouse model of DS and may play a role in the neuronal and behavioral deficits observed in these animals [38]. Indeed, behavioral deficits can be rescued in Ts65Dn mice by restoring SYNJ1 to disomy [39]. Further, also in DS mouse models overexpressing SYNJ1, several studies show cognitive dysfunction, early endosome enlargement and altered synaptic function [24,39,40]. These features closely mimic observations in DS autopsy studies showing endosomal enlargement that appears to be associated with the earliest A $\beta$  deposition with age [41-43]. In contrast, knocking out SYNJ1 in mice leads to premature death, along with neurological dysfunction and abnormal synaptic function [23], thus lower levels of SYNJ1 in sporadic AD may be partially involved with cognitive dysfunction.

Chromosome 21 also contains the gene for DRYK1a, which can phosphorylate SYNJ1 at multiple sites [44], but the resulting link between these two proteins has yet to be established in DS brain. Oligomeric forms of A $\beta$ , typically observed in AD brain, can destabilize SYNJ1 and leave individuals with DS with excessive levels of SYNJ1 vulnerable to A $\beta$  toxicity [45]. However, increased expression of SYNJ1 in older adults with DS and AD relative to DS alone may be beneficial or predict the development of AD. Despite the presence of significant AD neuropathology in DS by the age of 40 years, the presence of dementia is typically delayed until after 50 years [5].

The results of the current study provide several novel insights. SYN decreases in DSAD as it does in sporadic AD and to approximately the same extent. Further, decreased SYN in DS is associated with increased A $\beta$  neuropathology. SYN protein losses in the DS brain with AD may be linked to reports of neuronal loss [46-51] observed with age and with the presence of AD in these individuals. However, these reports are in brain regions including the entorhinal cortex and locus coeruleus with less information available on the frontal cortex. In contrast, SYNJ1 is consistently overexpressed in DS and may be an important component of synaptic function that leads to developmental cognitive deficits. DSAD is associated with higher SYNJ1 levels than DS alone and further associated with higher levels of SDS and FA extracted A $\beta$  1-40 and A $\beta$  1-42. The positive correlation between SYNJ1 and A $\beta$  independently of age at death suggests one possibility; higher A $\beta$  leads to reduced turnover of SYNJ1 in DSAD. The functional consequences of increased SYNJ1 in DSAD compared with DS may be twofold. First, given there is a 10 year delay between age of onset of AD



neuropathology and signs of dementia [5], higher SYNJ1 may be beneficial as hypothesized for other proteins that are overexpressed due to trisomy 21 [52]. However, it is important to consider the multiple genes that are present in triplicate in DS and the many proteins that are overexpressed in DS can have multiple and interacting detrimental and beneficial effects on neuronal function. Further, sporadic AD is also associated with a > 10 year prodromal phase that as of yet, has not been linked to SYNJ1 [53]. Higher SYNJ1 in DSAD compared to DS may also be detrimental, and as discussed previously, transgenic mice overexpressing SYNJ1 show abnormal neurological function [24,39,40].

The current study of aging and AD neuropathology in DS shows that SYN protein losses occur in DSAD as it does in sporadic AD. Interestingly, SYNJ1, which is overexpressed in DS is higher with the presence of AD neuropathology in DS. Given the significant number of genes on chromosome 21 that involve multiple synapse associated pathways, this should be a very interesting area to explore that may lead to novel interventions.

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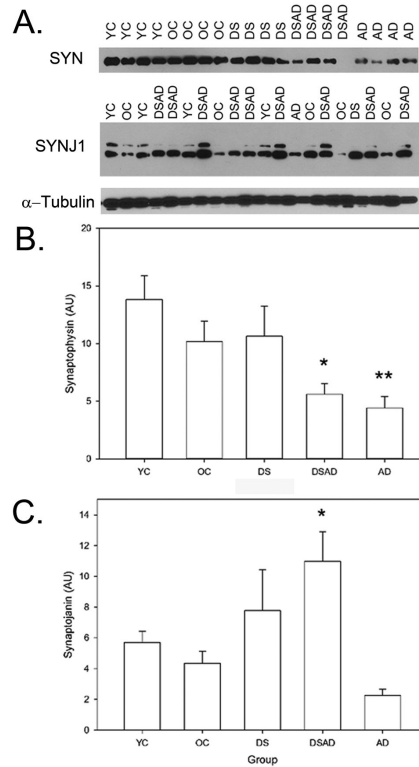
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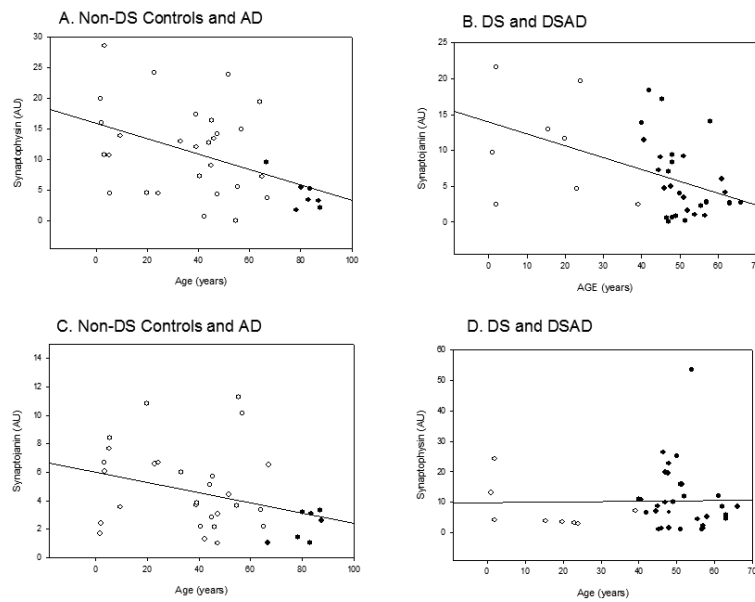
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**Figure 1.** SYN and SYNJ1 in DS. Representative western blots with SYN and SYNJ1 in YC, OC, DS, DSAD, and AD cases showing a ~38kDa and ~150 kDa bands, respectively.  $\alpha$ -tubulin was used as a loading control and did not show systematic differences predicting SYNJ1 or SYN protein levels (A). SYN was significantly lower in DSAD and sporadic AD cases relative to control or DS cases alone. DSAD was not different from sporadic AD in SYN level. \* $p < .05$  DSAD < DS, YC, OC, \*\* $p < .05$  AD < DS, YC, OC. (B). SYNJ1 was significantly higher in DS overall and further increased in DSAD. DSAD cases had significantly higher levels of SYNJ1 than sporadic AD cases. \* $p < .05$  DSAD > AD, OC. Bars represent group means and error bars are standard error of the mean.



**Figure 2.**

Changes in SYN and SYNJ1 with age. (A). Cases without DS showed a progressive decrease in SYN with age (open circles), with AD cases (closed circles) who were the oldest of the autopsy cases showed the lowest SYN overall. (B). In DS (open circles) and DSAD (closed circles), a similar significant decrease in SYN with age is observed. (C). SYNJ1 showed a progressive but not significant decrease with age in control cases (open circles) with AD cases (closed circles) being lower overall. (D). No age related changes were observed in SYNJ1 in DS (open circles) and DSAD (closed circles). Open circles are individual YC, OC, DS and DSAD cases. Closed circles are sporadic AD cases. Lines represent the linear regression.

**Table 1**

## Characteristics of Autopsy Cases

Characteristic	Control <40 years	Control >40 years	DS	DSAD	AD
	n=13	n=15	n=8	n=31	n=7
	Mean (Range)				
Age (yrs)	17.8 (1-39)	51.5 (41-67)	15.8 (1-39)	51.4 (40-66)	80.9 (67-88)
PMI (hrs)	16.2 (6-28)	14.9 (3-28)	17.0 (12-28)	7.6 (2-25)	8.4 (6-10)
	Number (%)				
Female (%)	5 (38)	8 (53)	2 (25)	18 (58)	2 (29)

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**Table 2**

SYN and SYNJ1 as a function of genotype and age group.

Synaptophysin *				
	Age Group	<40 years	>40 years	Total
Genotype	CTL	13.8 (7.4) (n=13)	10.2 (6.9) (n=15)	11.9 (7.3) (n=28)
	DS	10.6 (7.4) (n=8)	5.6 (5.1) (n=31)	6.6 (5.9) (n=39)
	Total	12.6 (7.4) (n=21)	7.1 (6.1) (n=46)	8.8 (7.0) (n=67)
Synaptojanin 1 *				
	Age Group	<40 years	>40 years	Total
Genotype	CTL	5.7 (2.6) (n=13)	4.3 (3.0) (n=15)	5.0 (2.9) (n=28)
	DS	7.8 (7.5) (n=8)	11.0 (10.7) (n=31)	10.3 (10.1) (n=39)
	Total	6.5 (5.0) (n=21)	8.8 (9.5) (n=46)	8.1 (8.3) (n=67)

\* Mean (Std Dev)

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**Table 3**Correlations between A $\beta$  and synapse proteins in DS cases (corrected for age).

A $\beta$	Synaptophysin	Synaptojanin 1
Oligomers	r=-0.008 p=0.95	r=0.13 p=0.3
PBS A $\beta$ 40	r=-0.23 p=0.07	r=0.24 p=0.06
SDS A $\beta$ 40	r=-0.20 p=0.13	r=0.24 p=0.05*
FA A $\beta$ 40	r=-0.23 p=0.07	r=0.27 p=0.03*
PBS A $\beta$ 42	r=-0.16 p=0.21	r=0.08 p=0.6
SDS A $\beta$ 42	r=-0.25 p=0.05*	r=0.28 p=0.03*
FA A $\beta$ 42	r=-0.22 p=0.09	r=0.27 p=0.03*

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