Title

Reduced synaptic proteins and SNARE complexes in Down syndrome with Alzheimer's disease and the Dp16 mouse Down syndrome model: Impact of APP gene dose

Journal

Alzheimer's & Dementia, 19(5)

ISSN

1552-5260

Authors

Chen, Xu-Qiao

Zuo, Xinxin

Becker, Ann

et al.

Publication Date

2023-05-01

DOI

10.1002/alz.12835

Peer reviewed

HHS Public Access

Author manuscript

Alzheimers Dement. Author manuscript; available in PMC 2024 May 01.

Published in final edited form as:

Alzheimers Dement. 2023 May ; 19(5): 2095–2116. doi:10.1002/alz.12835.

Reduced synaptic proteins and SNARE complexes in Down syndrome with Alzheimer's disease and the Dp16 mouse Down syndrome model: impact of *APP* gene dose

Xu-Qiao Chen[1,*], **Xinxin Zuo**[1], **Ann Becker**[1], **Elizabeth Head**[2], **William C Mobley**[1,*]

[1]Department of Neurosciences, University of California San Diego, La Jolla, CA 92093, USA

[2]Department of Pathology & Laboratory Medicine, University of California Irvine, Irvine, CA 92697, USA

Abstract

Introduction: Synaptic failure, a hallmark of Alzheimer's disease (AD), is correlated with reduced levels of synaptic proteins. Though people with Down syndrome (DS) are at markedly increased risk for AD (AD-DS), few studies have addressed snapse dysfunction.

Methods: Synaptic proteins were measured in the frontal cortex of DS, AD-DS, sporadic AD cases, and controls. The same proteins were examined in the Dp16 model of DS.

Results: A common subset of synaptic proteins were reduced in AD and AD-DS, but not in DS or a case of partial trisomy lacking triplication of *APP* gene. Pointing to compromised synaptic function, the reductions in AD and AD-DS were correlated with reduced SNARE complexes. In Dp16 mice reductions in syntaxin 1A, SNAP25 and the SNARE complex recapitulated findings in AD-DS; reductions were impacted by both age and increased *App* gene dose.

Discussion: Synaptic phenotypes shared between AD-DS and AD point to shared pathogenetic mechanisms.

Keywords

Down syndrome; Alzheimer's disease; partial trisomy 21; Dp16; synaptic protein; SNARE complex; APP; aging

[*]**Corresponding authors:** Xu-Qiao Chen, Department of Neurosciences, University of California San Diego, School of Medicine, 9500 Gilman Drive, GPL 341, La Jolla, CA 92093, USA. q0chen@ucsd.edu; William C.Mobley, Department of Neurosciences, University of California San Diego, School of Medicine, 9500 Gilman Drive, GPL 359, La Jolla, CA 92093, USA. wmobley@ucsd.edu.

AUTHOR CONTRIBUTIONS

X.-Q.C. and W.C.M. designed the research and W.C.M. supervised it. X.-Q.C., X.Z. and A.B. performed the experiments. X.-Q.C., E.H. and W.C.M. wrote the paper. All authors reviewed the paper and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All the procedures related to human samples were carried out under a protocol reviewed and approved by the human subjects review board at University of California San Diego (IRB #: 180620). All the animal studies were performed strictly according to the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. All experiments involving the use of animals have been approved by the University of California San Diego Institutional Animal Care and Use Committee (Protocol #: S09315).

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

1 NARRATIVE

1.1 Contextual background

Down syndrome (DS), due to trisomy for all or part of human chromosome 21 (HSA21), is associated with both developmental and age-related differences. Children are impacted by varying degrees by congenital heart disease, digestive tract disorders, hypothyroidism, disorders of the hematologic system including leukemia, disorders of sleep, and deficits in vision and hearing. Intellectual disability is essentially universal in children with DS; cognitive measures typically place them in the moderately severely affected range [1-3]. Differences in brain growth and function, including deficits in neurogenesis, neuronal migration, and maturation, are correlated with cognitive deficits and behavioral changes in children and young adults [4, 5]. Which changes contribute to cognitive deficits is uncertain, but reductions in neurogenesis, neuronal number, dendrite morphology, and dendritic spines have been reported [5-9]. Also, the synaptic protein SNAP25 was significantly reduced in fetuses with DS [10].

With surgical correction of congenital heart lesions together with improved general medical care and engagement in society, the life expectancy for those with DS is approximately 60 years [11]. Interestingly, cognitive function is stable over several decades in children and young adults with DS. However, in older adults, dementia is extremely common; indeed, people with DS have a significantly increased risk for Alzheimer's disease (AD), referred to herein as AD-DS. Dementia ensues on average in the mid-50s [12]. With increased longevity, AD-DS has increased considerably [13]. AD-DS is accompanied by significant reductions in neuron numbers in several brain regions, by dendritic atrophy, and by reductions in dendritic spines in visual cortex and hippocampus [14-17].

Given the biological complications induced by an extra chromosome and the marked differences relative to neurotypical individuals with respect to cognition in childhood, it is striking that those with AD-DS demonstrate many clinical and pathological features in common with sporadic and familial forms of AD. Clinical manifestations in DS are consistent with progressive cognitive dysfunction [1, 4, 12, 18] and clinical biomarkers are closely related to those in AD [19-21]. Amyloid plaque and neurofibrillary tangles are well-documented pathological hallmarks of both AD and AD-DS [2]. Though less well characterized, changes in endosomal-lysosomal pathways, reactive astrogliosis, and loss of neurons serve as hallmarks of both [2, 22]. Synaptic dysfunction is viewed as contributing to both the onset and progression of AD and synapse loss is well correlated with deficits in cognitive function [23, 24]. Although synaptic loss in AD-DS is not well documented, it is supported by rare reports of decreases in synaptic proteins [25-27].

Proteins present at synapses that mediate synaptic vesicle-mediated exocytosis of neurotransmitters are of particular interest in understanding synaptic function in degenerative disorders, including AD and AD-DS. Synaptic vesicles contain proteins whose coordinated functions enable the vesicle to support the extremely rapid release of neurotransmitters in response to locally increased calcium levels. In a process yet to be fully defined, exocytosis employs three protein machines. The *core fusion complex*

priming the synaptic vesicle for release is composed of synaptobrevin 2 (also called vesicle-associated membrane protein 2, VAMP2) on the synaptic vesicle, syntaxin 1 on the plasma membrane, and SNAP25; these three form the triple SNARE complex that together with Munc18-1 brings into apposition the membranes of the synaptic vesicle and the plasma membrane. Upon binding calcium, the *calcium sensor* synaptotagmin 1, located on the synaptic vesicle, binds to the *core fusion complex* and with complexin supports fusion of the vesicle and presynaptic membranes, causing neurotransmitter release [28]. The presynaptic *active zone protein complex,* containing RIM, Munc13, and RIM-BP and a calcium channel, coordinates vesicle docking, vesicle priming, and recruitment of the calcium channel to the docked vesicle. Other proteins contribute to synaptic vesicle structure and function. The synaptic vesicle protein 2 (SV2) protein family and synaptic vesicle membrane proteins like synaptophysin and synapsin may regulate the kinetics of synaptic vesicle fusion [29].

One approach to understanding the molecular underpinnings of synaptic dysfunction and loss is to examine brain levels of synaptic proteins. Their levels have been examined by immunoblotting and immunostaining in the AD brain. Several reports address measures of synaptic proteins in AD and mild cognitive impairment (MCI) showing reductions, albeit not entirely consistently, in several proteins located pre- and post-synaptically [24, 26, 30-38]. Given the similar pathologies for AD and AD-DS, the AD-DS brain would be predicted to show the same changes. But much less is known of the levels of these proteins in the DS brain. Indeed, our review of the literature showed few papers describing on synaptic proteins loss in AD-DS and comparing DS to AD-DS [25-27, 39], thus a systematic approach is needed to document the change of synaptic proteins in DS. Studies in DS thus offer the opportunity to explore synaptic markers and to ask to what extent changes are present before and after the emergence of AD-DS. Accordingly, in the frontal cortex of adult DS and AD-DS brains, using quantitative immunoblotting we addressed the following questions: 1) are there synaptic changes in adults with DS and/or AD-DS; and if so, 2) by what means does the extra chromosome 21 induce them; 3) do they recapitulate those in AD; and 4) is the underlying mechanism(s) in AD-DS the same as in AD. Answers to these questions could elucidate the pathogenesis of AD in those with DS and provide a point of departure for interventions to prevent AD-DS as well as AD.

Complementing studies on synapses are studies addressing synaptic markers as fluid biomarkers. The identification of fluid biomarkers whose changes can be correlated with cognitive status are enabling clinicians to better understand and diagnose AD and may do so for AD-DS. They enable clinicians to characterize the disease status of individual patients and to design clinical trial cohorts optimized for the safety and efficacy of interventions. Fluid biomarkers have now been defined for both AD and AD-DS. Those that report the status of synapses and axons may prove most useful. Neuronal pentraxin 2 (NPTX2) is a synapse-derived marker that is evaluated in cerebrospinal fluid (CSF) of those with AD [40] and AD-DS [41]. Neurofilament L (NfL), exclusively expressed in neurons and enriched in axons, has been measured in plasma and CSF in several neurodegenerative disorders [42, 43] and is increased in AD. Increases in NfL are also seen in those with DS as early as the mid-30s - i.e. before the onset of dementia - and levels further increase with age and the diagnosis of AD-DS [20, 44]. The two isoforms of the synaptic protein syntaxin, syntaxin

1A and syntaxin 1B, share more than 80% amino acid homology and are coexpressed in neurons [45]. Syntaxin 1B is also evaluated as a CSF biomarker for AD [46].

1.2 Study design and main results

We examined synaptic proteins in the frontal cortex of AD-DS, DS, and age-matched neurologically healthy controls, focusing on the *core fusion complex* (syntaxin 1A, synaptobrevin 2, and SNAP25), the *calcium sensor* synaptotagmin, synaptic vesicle proteins (SV2A, synaptophysin and synapsin 1) and on PSD95, the latter marking the postsynaptic membrane of excitatory synapses. We also examined the levels of preformed SNARE complexes. To compare DS and AD-DS with AD, we independently analyzed the same proteins and SNARE complexes in frontal cortex. Quantitative immunoblotting was used to define levels. Correlational analysis evaluated potential age- and postmortem interval (PMI)-dependent changes. In AD-DS and AD we found decreases in levels of several synaptic proteins, including SNARE proteins syntaxin 1A and SNAP25, synaptic vesicle proteins synaptophysin and synapsin 1, and PSD95. In addition, SNARE complexes were reduced. Remarkably, with one exception, decreases in synaptic proteins were present in AD-DS but not DS samples.

To explore the mechanism(s) underlying changes in synaptic proteins, we examined the frontal cortex of a rare case of partial trisomy (PT-DS) [47] in whose genome only two copies of the *APP* gene were present. Studies in this patient allowed us to explore whether or not *APP* gene dose is required for the reduction of synaptic proteins. Interestingly, with the exception of PSD95, the PT-DS cortex displayed a normal profile of synaptic proteins as well as normal levels of the SNARE complex. To further explore the biology of synaptic loss in DS we examined synaptic proteins and SNARE complexes in the cortex of the Dp16 mouse model of DS. Prior studies in the brain of the Ts65Dn mouse model of DS showed reductions in synaptophysin and syntaxin 1 [48, 49]; findings were not consistently reproduced [50]. The Dp16 model harbors a duplication of mouse chromosome 16 genes whose homologues are present on the long arm of HSA21. Unlike the Ts65Dn mouse, the Dp16 mouse carries a third copy of all mouse 16 genes present on HSA21 and no extra copy of mouse genes whose homologues are not on HSA21. The Dp16 mouse thus more faithfully replicates the genetic constitution of those with DS and, thereby, enables studies to more specifically explore molecular and cellular mechanisms in DS [51]. Dp16 displays age-related features of the neuropathology in an *App* gene dose-dependent manner including neuronal loss, phosphorylation of tau, endosomal changes, and activation of astrocytes [52]. To test for the impact of increased *App* gene dose, Dp16 mice were crossed with 2N mice lacking one copy of *App* (i.e., 2N: *App*+− mice) on the same strain background to produce Dp16 mice harboring only two mouse *App* alleles (i.e., Dp16: *App*++−). In Dp16 mice, we saw reductions in syntaxin 1A, SNAP25, and in SNARE complexes. Both age- and *App* gene dose-mediated changes were detected. Reductions were apparent by age 9-10 months and required increased *App* gene dose.

Finally, we addressed cortical levels of proteins used as clinical biomarkers, including NfL, NPTX2 and syntaxin 1B, in frontal cortex in AD-DS, DS and in Dp16 mouse cortex. All

were reduced in AD-DS but not in DS; only very old Dp16 mice showed reductions in NPTX2 (20 months).

1.3 Study conclusions, potential clinical applications, and other implications

The developmental brain changes in DS raised the possibility that synaptic markers would differ throughout the lifespan. To explore this question, we examined the levels of synaptic proteins in DS brains from individuals without and with the diagnosis of AD. Significantly, and to some extent unexpectedly, there were no reductions in DS participants not diagnosed with dementia, indicating that changes in synaptic proteins in frontal cortex were not a function of developmental differences. The one exception was for a reduction in PSD95 in males with DS. Thus, only in AD-DS did we find reductions in both the presynapse and postsynapse proteins; reductions included components of the SNARE complex (syntaxin 1A, SNAP25), synaptic vesicle proteins synaptophysin and synapsin 1 as well as PSD95. Evidence for the significance of the decreases in SNARE proteins was the finding that preformed SNARE complexes were also reduced in the AD-DS brain. Moreover, within the AD-DS cohort synaptotagmin showed age-related decreases, suggesting that this calcium sensor also marks synapse dysfunction in the eldest AD-DS patients. Using the same methods as for AD-DS we found that the changes in synaptic proteins in AD-DS were recapitulated in the AD brain.

Our findings allow initial responses to the questions raised above. First, the data point to synaptic dysfunction or loss in the frontal cortex of adults with DS, but only in those diagnosed with dementia. Given that younger individuals with DS do not show reduced levels of synaptic proteins, we can suggest that other mechanisms contribute to the developmental cognitive phenotype, at least as regards frontal cortex mediated functions. Second, studies in the PT-DS case and the mouse model point to increased *APP* gene dose as necessary for reductions in synaptic proteins and SNARE complexes. It appears, then, that increases in APP and its products are necessary not only for the pathological hallmarks and cognitive loss that characterizes AD-DS but also for the synaptic dysfunction or loss. Third, the changes in AD-DS mirror those in AD, drawing an important parallel between these disorders. Fourth, the reductions in synaptic protein shared in AD and AD-DS offer further compelling support that these disorders also share in molecular and cellular events leading to synaptic dysfunction and loss.

Increased *APP* gene dose results in increased levels of full-length APP and its products [47, 52]. The necessity for increased *APP* gene dose for AD-DS and for reduced synaptic proteins in AD-DS is complemented by studies in the Dp16 mouse model in which we showed that increased *App* gene dose was also necessary for tau pathology, changes in early endosomes, astrocytes and microglia and for neuronal loss [52]. It is intriguing to speculate that tau pathology at the synapse [53] may compromise synapse structure and function, as has been suggested [54]. The impact of increased APP products in DS may also inform the discussion regarding the molecular basis for AD in the non-DS population, most obviously the critical role for increased *APP* gene dose in some types of familial AD (FAD) due to *APP* gene duplication [55]. Also, the evidence that FAD mutations in presenilin contribute to pathogenesis through increased levels of longer Aβ peptides (i.e. those ≥42 residues) [56].

Taken together, these observations strongly support a central role for the dose of the *APP* gene and its products in the pathogenesis of AD-DS as well as AD.

1.4 Limitations, unanswered questions, and future directions

Given the availability of tissue it is noteworthy that the number of cases available for study was limited, with 12 for DS and 11 for AD-DS. While adequate to support the main conclusions, the numbers of male (DS: n=6; AD-DS: n=7) and female (DS: n=6; AD-DS: n=4) cases constrain our ability to interpret some of the sex-related differences in the brain levels we found. Even given small numbers, the magnitude of the decreases in NfL in both DS and AD-DS males versus females is striking. In DS the significant decrease in PSD95 in males, but not females, may also reflect a true sex difference. Studies of age-related changes are limited when samples span a narrow range of ages. Addressing this concern our samples for DS spanned 40 years and for AD-DS 24 years. We found no age-related changes in any DS sample and in AD-DS only three such changes (SNAP25, synaptotagmin and NfL). Given progressive increases in plasma NfL with age in the DS population beginning at about age 45, the absence of changes in brain levels in the non-demented DS cohort was a surprise. We speculate that a larger number of samples may have allowed us to detect more age-related differences but doubt that it would have given a different overall conclusion.

A second limitation was that our studies examined only frontal cortex. Developmental changes differentially impact DS brain regions (for review see [5]). Moreover, there are significant regional differences in timing and severity of pathological changes in AD-DS [2, 14, 17, 57, 58]. While existing data show that cortical neuron number is impacted both developmentally [5, 6, 8], and in AD-DS [2, 57] this region appears to be impacted later than subcortical regions (locus coeruleus, nucleus basalis), entorhinal cortex and certain hippocampal regions [57] and to a lesser extent [14, 17, 58]. Accordingly, our findings distinguishing DS and AD-DS may have shown differences if we had examined entorhinal cortex or hippocampus. Note however, that those in DS group were judged to be cognitively stable, indicating that any such changes recorded may have reflected developmental and not degenerative differences.

By definition, studies in postmortem brain provide only a snapshot of measures of interest, in our case the levels of synaptic proteins. More informative would be longitudinal studies that speak to synapse structure and function. One approach would employ human model systems to assess synaptic structure and function over time. As one example, it is possible to create organoids that contain human iPSC-derived neurons, and with technical advances they may also be engineered to contain human glial cells, including microglia and blood vessels [59], thus creating more realistic models. A caveat is that organoids kept *in vitro* demonstrate properties of the young brain, but this limitation appears to be answered by transplanting organoids to the mouse brain wherein maturation to the adult stage occurs [60]. Serial investigations of DS organoids would allow age-related changes in synapse structure and function in a truly human context. It would also support studies exploring the impact of increased *APP* gene dose and the discovery of which APP product(s) contributes to synaptic compromise.

Complementing studies in human models, longitudinal measures of fluid biomarkers reflecting synapses could prove quite useful. Clinical biomarkers are increasingly examined in DS cohorts [61, 62]. Fluid biomarkers that demonstrate a concurrence between brain and fluid measures may prove highly informative. To date, reduced levels of classical synaptic proteins in the AD-DS brain are correlated only with an increase in SNAP25 in the CSF [21]. Our studies point to decreases in NfL, NPTX2 and syntaxin 1B in the frontal cortex of those AD-DS, but not DS. However, those with DS that are not demented also have increased NfL in CSF and plasma and decreased CSF NPTX2. These findings suggest that no simple formula exists that links the levels of synaptic proteins in frontal cortex and in fluid biomarker for these proteins.

The importance of synaptic failure and loss in AD and AD-DS motivates studies to robustly increase efforts to evaluate synaptic proteins. In part, this can be served by postmortem studies in larger cohorts whose clinical history and biomarker status are more thoroughly detailed. In like manner, additional longitudinal studies of fluid biomarkers for synaptic proteins would further define changes in existing biomarkers and discover new biomarkers. Also useful will be studies of imaging biomarkers for synapse structure and function [63] that could be used to inform and interpret fluorodeoxyglucose (FDG)-positron emission tomography (FDG-PET) data for glucose utilization and studies of tissue regional volumes by magnetic resonance imaging (MRI). The utility of an increasing set of predictive synaptic biomarkers will take on increased importance as preclinical studies identify promising therapies for clinical trials in the DS population.

2 CONSOLIDATED RESULTS AND STUDY DESIGN

Synapse loss features prominently in the AD brain and is correlated with cognitive deficits. To ask if the same correlations are found in demented adults with DS (AD-DS), and to compare findings for synaptic proteins in AD-DS with those in AD, we examined synaptic proteins and the levels of SNARE complexes that mediated neurotransmitter release. A total of nine synaptic proteins were quantitated using immunoblotting in the frontal cortex, comparing DS, AD-DS and AD with age-matched controls. Five synaptic proteins were significantly reduced in AD-DS and AD, but not in DS. In both AD-DS and AD reductions in two of the components of the SNARE complex, syntaxin 1A and SNAP25, were accompanied by significant reduction in the preformed SNARE complexes in which they participate. The essential role played by the SNARE complex in neurotransmission is evidence that the reductions in protein levels is correlated with synapse dysfunction in both AD-DS and AD. That neither synaptic proteins nor SNARE complexes were significantly impacted in non-demented adults with DS supports the view that synapse dysfunction or loss contributes to dementia in AD-DS. Moreover, shared reductions in synaptic proteins and SNARE complexes in AD-DS and AD are evidence for similar pathogenetic events and possibly for shared pathogenetic mechanisms.

To explore mechanisms underlying reductions in synaptic proteins studies, the frontal cortex from an elderly partial trisomy 21 (72 years)(PT-DS), whose genome carries only the normal two copies of the *APP* gene and who showed no evidence of dementia or AD pathology [47], was used explore the possible role of *APP* gene dose on synaptic proteins. Compared

with cognitively normal controls, with the exception of PSD95 all the synaptic proteins were normal as was the levels of the SNARE complex. The PT-DS case thus showed the same profiles of synaptic proteins as did males with DS that were not demented. To explore mechanisms underlying synaptic protein loss in AD-DS we carried out studies in the Dp16 mouse model of DS. The same synaptic proteins were examined in cortex, comparing the values in Dp16 mice with age-matched euploid mice (i.e. 2N) mice. Recapitulating key findings in AD-DS and AD, there were reductions in SNARE complex proteins syntaxin 1A and SNAP25 and in the SNARE complex. These changes were first detected in mice 9-10 months old and persisted through 20 months of age. In view of the necessity for increased *APP* gene dose for AD-DS, and findings in the same mouse model of DS demonstrating the necessity of increased *App* gene dose for AD-related pathological changes, we asked if normalizing *App* gene dose in Dp16 mice (Dp16: *App*++−) would prevent synaptic changes. Dp16 mice were crossed with 2N mice lacking one copy of *App* (i.e., 2N: *App*+− mice) on the same strain background to produce the Dp16: *App*++− mice. In so doing we discovered that the reductions of SNARE proteins and preformed SNARE complexes was prevented.

We extended our studies to evaluation of brain levels of axonal and synaptic proteins that may serve as clinical fluid biomarkers for assessing disease status in DS. In AD-DS, but not DS, we detected reductions in the levels of NfL, NPTX2 and syntaxin 1B. Findings in the Dp16 mouse demonstrated reductions in NPTX2, but only in very old mice (20 months old).

Our findings are evidence for synaptic changes that distinguish demented adults with DS (AD-DS) from those not diagnosed with dementia, thereby drawing a compelling correlation between synaptic protein reductions and the presence of cognitive deficits that characterize AD-DS. The evidence for shared changes in synaptic proteins and SNARE complexes between AD-DS and AD raises the possibility of shared mechanisms. Toward the goal of elucidating mechanisms, studies in the PT-DS patient and the Dp16 mouse point to a role for increased *APP* gene dose for synaptic changes, suggesting a role for APP and its products in the pathogenesis of AD-DS and AD.

3 DETAILED METHODS AND RESULTS

3.1 METHODS

3.1.1 Antibodies—Mouse antibody anti-syntaxin 1A (1:5000; S0664; HPC-1) and rabbit antibody anti-APP (1:3000; A8717) were obtained from MilliporeSigma (Burlington, MA); rabbit antibody anti-SNAP25 (1:2000; 14903-1-AP), anti-NPTX2 (10889-1-AP), mouse antibodies anti-synaptophysin (1:5000; 67864-1-Ig), anti-β-actin (1:10,000; 60008-1-Ig) were from Proteintech (Rosemont, IL). Mouse antibody anti-synaptobrevin 2 (1:5000; 104 211), rabbit anti-neurofilament L (171 002), and anti-syntaxin 1B (110 402) were purchased Synaptic Systems (Goettingen, Germany). Mouse antibody anti-synaptotagmin (1:2000; 610434) was from BD Biosciences (Franklin Lakes, NJ). Rabbit antibodies anti-synapsin 1 (1:5000; 5297) and anti-synaptojanin 1 (1:1000; 80377) were got from Cell Signaling Technology (Danvers, MA). Mouse antibody anti-SV2A (1:5000; sc-376234) was obtained from Santa Cruz Biotechnology (Dallas, TX). Mouse antibody anti-PSD95 (1:2000; 7E3-1B8) was from ThermoFisher Scientific (Waltham, MA). In all cases, a single band or in some cases doublet, at the expected molecular weight was quantitated. Bands

at other molecular weights, of uncertain identify, were detected with some antibodies; they were usually of lesser intensity and were not included in our analysis.

3.1.2 Human samples—Human DS, AD-DS, and control frontal cortexes (male and female) (DS, n = 12, age 22–62; AD-DS, n = 11, age 41–65; and control for DS (C/DS), n = 10, age 46-57; control for AD-DS (C/AD-DS), n = 12, age 39-59) were obtained from NIH NeuroBioBank and the Alzheimer Disease Research Center (ADRC) at UCI MIND (Table 1). Human AD cortexes (AD, n = 14, age 64-100; control for AD (C/AD), n = 12, age 58-103) were received from the Neuropathology/Brain Bank for ADRC at UCSD and Banner Sun Health Research Institute. One rare DS case with partial trisomy 21 as reported previously [47] was also included in this study. The cognitive status of those with DS (AD-DS) and AD was listedin the samples provided by the NIH NeuroBioBank, UCI, Banner Sun Health Research and ADRC (UCSD). As published [64] human samples of approximately 10 to 20 mg frozen tissue were processed in 0.5 ml RIPA buffer (50 mM Tris-HCl pH 8.0, 150 mM NaCl, 1% NP-40, 1% Triton X-100, 0.1% sodium deoxycholate, 0.1% sodium dodecyl sulfate and protease inhibitor cocktail [4693124001; Millipore-Sigma]) and rotated in 4 °C for 30 min. All lysates were bench centrifuged (12,000 rpm for 15 min at 4 °C) to produce supernatants, and the protein content of supernatants of each sample was determined using the Bradford assay (5000001, Bio-Rad, Hercules, CA). Samples were examined using Western blotting analysis.

3.1.3 Dp16 mouse model of DS—Dp (16)1Yey/+ (Dp16) mice were obtained from the Jackson Laboratory (stock #: 013530). Dp16 mice contain a duplication orthologous to human chromosome 21q11-q22.3 and carry 113 genes orthologous to genes on HSA21. Dp16 mice were maintained by crossing females to male (C57BL/6J × C3H/HeJ) F1 mice (B6C3). Diploid (2N) littermate mice on the same background served as controls. To produce Dp16 mice harboring only two mouse *App* alleles (i.e., Dp16: *App*++−), Dp16 mice were crossed with 2N mice lacking one copy of *App* (i.e., 2N: *App*+− mice) on the same strain background. The genotype of all animals was confirmed by polymerase chain reaction (PCR). For genotyping, tail samples were used to extract genomic DNA. A protocol was established to amplify the HPRT insertion, which is only found in Dp16 mice along with amplification of the IL-2 gene as an internal control. The primer sequences used for HPRT were: fwd: 5’-AGGATGTGATACGTGGAAGA-3’; rev: 5’-CCAGTTTCACTAATGACACA-3’; while the primers for IL-2 were: fwd: 5'-CTAGGCCACAGAATTGAAAGATCT-3’; rev: 5'-GTAGGTGGAAATTCTAGCATCATCC-3’. For m*App* the primers were fwd: 5’-AGAGCACCGGGAGCAGAGCG-3’; rev: 5’-AGCAGGAGCAGTGCCAAGC-3’. For the *Neo* insert the primers were: fwd: 5’-ATGGATACTTTCTCGGCAGGAGC-3’; rev: 5’-GAGGCTATTCGGCTATGACTGGG-3’. All animals were maintained and bred according to standard procedures. Mice were housed two to five per cage with a 12-hour light-dark cycle and *ad lib* access to food and water. Mice for all studies used sample sizes targeted to detect statistically significant differences of ≥20%.

3.1.4 Western blotting—Equal amounts of total proteins for each sample (10–20 μg) were separated on SDS-PAGE and then electro-transferred to PVDF membranes (Bio-Rad).

The membranes were blocked with 5% nonfat milk for 1 h and probed with specific primary antibodies overnight at 4 °C followed by incubation with goat anti rabbit IgG-HRP (1:15,000; 111-035-144) or anti-mouse IgG–HRP (1:15,000; 115-035-003) (Jackson ImmunoResearch Laboratories; West Grove, PA) at room temperature for 1 h. SDS-resistant SNARE complexes were analyzed using non-boiled samples loaded into the same SDS-PAGE gel and probed with anti-syntaxin 1, SNAP25 and synaptobrevin 2 antibodies, as above. All blots were developed using the BioRad Clarity Western ECL substrate and captured using ChemiDoc XRS + (Bio-Rad); only blots within signals in the linear range were quantitated using the ImageLab 3.0.1 software (Bio-Rad).

3.1.5 RNA isolation and quantitative PCR—Total RNA was extracted from the cortex of 2N, Dp16 and Dp16: *App*++− or from AD-DS, C/AD-DS, DS, C/DS frontal cortexes using TRIzol (Invitrogen) and the Direct-zol RNA miniprep kit (Zymo Research) and equal amounts of total RNA were used for cDNA generation with iScript™ cDNA Synthesis Kit (Bio-Rad) following the manufacturer's instructions. The primer sequences used for syntaxin 1A in mouse were fwd: 5'-AGAGATCCGGGGCTTTATTGA, rev: 5'-AATGCTCTTTAGCTTGGAGCG; SNAP25: fwd: CAACTGGAACGCATTGAGGAA, rev: 5'-GGCCACTACTCCATCCTGATTAT, both from PrimerBank [65]. The primer sequences for human NPTX2 were fwd: 5'-CATCGAGCTGCTCATCAAC, rev: 5'-CTGCTCTTGTCCAAGGATC [40]. qPCR was for 40 cycles. Endogenous β-actin mRNA was used as the internal control with the sequences: 5'-GATCATTGCTCCTCCTGAGC; rev: 5'-ACATCTGCTGGAAGGTGGAC. Values within the log-linear phase of the amplification curve were defined for each probe/primers set and analyzed using the ΔΔCt method (Applied Biosystems 7300 Real-Time PCR System).

3.1.6 Statistic analysis—All data are presented as the mean ± SEM. Statistical analyses were performed using PRISM (GraphPad Software Inc., La Jolla, CA) with a two-tailed Mann-Whitney test (human samples) or student *t*-test (mouse samples due to smaller size and variability) or one-way ANOVA test followed by Newman-Keuls Multiple Comparison Test. The significance levels were *P < 0.05, **P < 0.01, and ***P < 0.001.

3.2 RESULTS

3.2.1 Reductions in specific synaptic proteins in the frontal cortex of those with AD-DS and AD but not DS—To evaluate the status of synapses in AD-DS, we measured the levels of a comprehensive list of synaptic proteins. Of particular interest were proteins that contribute to the *core fusion complex* and the *calcium sensor,* synaptotagmin. We measured the SNARE complex proteins syntaxin 1A, SNAP25, and synaptobrevin 2, as well as the calcium sensor synaptotagmin in the frontal cortices of AD-DS subjects, comparing the values for those in age-matched controls (C/AD-DS) (Table 1). Syntaxin 1A and SNAP25 were significantly reduced in the AD-DS brain. Synaptobrevin 2 levels varied greatly, but synaptobrevin 2 was not significantly reduced, nor was synaptotagmin (Figure 1A, 1D). To explore sex-related differences, we compared the levels in males and females versus their controls. Reductions in syntaxin 1A were significant in both males and females; for SNAP25, only the decrease in females was statistically significant (Supplemental Table 1).

Synaptophysin and synapsin 1 are routinely used to mark the presynapse; PSD95 is a marker of postsynaptic membranes. The levels of these proteins in brain homogenates serves as an indirect measure of synapse number. In comparison to controls, all three proteins were significantly reduced in AD-DS brains (Figure 1A, 1D). Synapsin 1 levels were reduced significantly in both females and males. For synaptophysin, examining females and males separately we found significant reductions in males and trends to reductions in females; the same pattern applied to reductions in PSD95 (Supplemental Table 1). SV2A is a synaptic vesicle protein whose function is yet to be fully defined but includes regulation of neurotransmitter release [66]. The levels of SV2A in samples varied and there was no significant reduction in AD-DS subjects (Figure 1A, 1D). Synaptojanin-1 is a phosphoinositide phosphatase that plays a role in endocytosis and synaptic vesicle recycling [67, 68]. Consistent with the presence of the gene for this protein (*SYNJ1*) on HSA21, the levels of synaptojanin 1 were increased in comparison to controls in both female and male AD-DS samples (Figure 1A, 1D; Supplemental Table 1). Interestingly, the 2- to 3-fold increases in synaptojanin-1 levels exceeded those predicted by gene dose alone. There was no significant difference in the age at death of AD-DS and C/AD-DS subjects, nor was there a significant difference in postmortem interval (PMI) (Table 1). PMI had no significant effect on the levels of the synaptic proteins significantly reduced in AD-DS subjects except for the levels of PSD95 in the C/AD-DS group (Supplemental Table 2).

Because age plays a defining role in the emergence of AD in DS, we also asked if there were age-related changes in the levels of synaptic vesicle proteins in AD-DS and C/AD-DS samples (Table 2). Patient age was significantly inversely correlated with the levels of only synaptotagmin and SNAP25 in AD-DS cortex, but not in C/AD-DS. We conclude that reductions in a select set of synaptic proteins important for neurotransmission are evidence of synaptic dysfunction or loss in AD-DS.

Earlier reports addressing synaptic proteins in AD documented changes in several synaptic proteins in different brain regions but with conflicting results [24, 26, 30-38]. To compare the findings for AD-DS to those in AD, we examined the levels of the same synaptic proteins in the frontal cortex of AD and age-matched controls (Supplemental Table 3). In contrast to earlier reports [33, 37], neither the levels of synaptotagmin nor synaptobrevin 2 were reduced in the samples examined herein. Consistent with our findings in AD-DS, there were reductions in syntaxin 1A, SNAP25, synaptophysin, synapsin 1 and PSD95 in AD samples (Figure 1C, 1D). When females and males were examined separately, we found that reductions in syntaxin 1A, SNAP25 and PSD95 were present in both sexes (Supplemental Table 1). Synaptophysin and PSD95 were significantly reduced in females with trends to reductions in males (Supplemental Table 1). Age at death did not distinguish the AD and C/AD samples while PMI, when recorded, of AD samples was slightly less than for C/AD samples (Supplemental Table 3). PMI had no effect on the levels of the synaptic proteins whose levels were reduced in AD samples (Supplemental Table 2), nor did age at death have such an effect (Table 2). We conclude that AD-DS shares with AD reductions in synaptic proteins present in both the presynaptic and postsynaptic compartments.

DS impacts both brain development and aging. It was therefore of interest to determine whether the changes in AD-DS were also present in those with DS without a diagnosis of

dementia. We examined brain samples from people with DS without AD, comparing them to their age-matched controls (C/DS) (Table 1). There was no significant difference in the levels of the components of the SNARE complex: syntaxin 1A, SNAP25, and synaptobrevin 2. The levels of synaptic vesicle proteins synaptophysin, synapsin 1, synaptotagmin and SV2A also failed to distinguish between DS and controls (Figure 1B, 1D). Interestingly, while there was no change in PSD95 in combined female and male samples, this protein was significantly reduced in males (Supplemental Table 1). As in AD-DS, synaptojanin 1 was increased in DS in both females and males (Figure 1B, 1D; Supplemental Table 1) a finding consistent with a previous report [25]. There was no significant difference in PMI in the DS and C/DS samples (Table 1). Age had no effect on the levels of synaptic vesicle proteins in DS or C/DS (Table 2).

Our DS samples included four male individuals whose age was younger than that for control cases, resulting in a significant difference in average ages (Table 1; C/DS versus DS). To exclude the possibility that including these young cases influenced the findings, we excluded them from the analysis and reanalyzed the data. The results were the same as for the entire group (Supplemental Figure S1A). The presence of only two males precluded separate statistical analysis of males; nevertheless, the decrease in PSD95 in males was again seen (Supplemental Table 1). The increase in synaptojanin 1 seen in AD-DS was also present and significant for the combination of females and males and for females alone, with a trend for males (Supplemental Figure S1A; Supplemental Table 1). These data are evidence that with the exception of PSD95 in males, the levels of synaptic proteins are not reduced in DS subjects prior to a diagnosis of dementia. We conclude that deficits in synaptic proteins accompany the emergence of dementia in people with DS.

3.2.2 Studies on the SNARE complex: reductions in AD-DS, AD, but not DS

—Studies reporting the levels of synaptic proteins in lysates of brain tissue record total levels but do not specifically capture those engaged in synaptic function. SNARE proteins SNAP25, syntaxin 1 and synaptobrevin 2 are present together in the SNARE complex that mediates vesicle release [69]. Measures of the SNARE complex directly address the levels of these proteins engaged in synaptic vesicle release. Therefore, to ask if the reductions in SNAP25 and syntaxin 1A resulted in reduced levels of the SNARE complex we assessed SNARE complexes in the frontal cortex of those with AD-DS and AD. To detect SNARE complexes, we used an established method in which complexes are solubilized in SDS but are not boiled [70, 71]. We found reduced levels of the SNARE complex in both AD-DS (Figure 2A-C) and AD (Figure 2G-I). Figure 2 reports the data when immunoblotting for syntaxin 1. Note that all samples demonstrated a ladder of bands corresponding to complexes of different apparent molecular weights, each of which contain syntaxin 1A (Figure 2A, 2G). The band at 73 kD is consistent with a complex containing a trimer consisting of a single molecule of syntaxin 1A, SNAP25, and synaptobrevin [70]. The bands at higher apparent molecular weights also contain syntaxin 1, pointing to its presence in higher order complexes. In addition to syntaxin 1, SNAP25 and synaptobrevin also contribute to the band at 73 kD and to the higher order complexes (Supplemental Figure S2). Note that when samples were boiled to disassemble complexes only the monomers of each protein are detected (Figure 2 and Supplemental Figure S2). AD-DS and AD samples both

showed significantly reduced levels of the SNARE complex in comparison to their controls (C/AD-DS and C/AD). Decreases were evident in the band at 73 kD (Figure 2A, 2C, 2G, 2I) as well as in the higher molecular weight species (Figure 2A, 2B, 2G, 2H). In contrast, there was no decrease in SNARE complex bands in the brains of those with DS without AD (Figure 2D, E, F). The reductions in the SNARE complex in AD-DS and AD are further evidence for compromised synaptic function. The preservation of SNARE proteins and SNARE complexes in DS but not AD-DS shows that AD-like changes in synaptic structure and function are correlated with the clinical diagnosis of dementia.

3.2.3 Impact of *APP* gene dose on synaptic proteins and SNARE complexes in DS—In those with DS, increased *APP* gene dose is necessary for amyloid plaque and tau pathology and for dementia [47, 52, 72]. It is also necessary for the changes in the levels of the full-length APP and its Aβ peptide products [47, 52]. These insights regarding *APP* gene dose is informed by studies of rare cases of partial trisomy 21 (PT-DS) in which the DS participant's genome carries only two copies of the *APP* gene. One such case is a man followed over several years who died at age 72 with no evidence for dementia; at postmortem exam his brain did not show the neuritic plaques and sufficient neurofibrillary tangles for a diagnosis of AD [47]. To ask if increased *APP* gene dose is also necessary for synaptic loss we examined the same set of synaptic proteins in a frontal cortex sample of the PT-DS patient, comparing the levels to those in cognitively normal controls. For our controls (C/PT-DS) we used only male samples and enriched for increased age at death; the average was ~56 years (Supplemental Table 4). Consistent with normal *APP* gene dose, the PT-DS sample showed normal levels of the full-length APP protein. Also consistent with his partial trisomic state and genetic mapping, there was an increase in the level of synaptojanin-1. Importantly, we found that the PT-DS sample displayed normal levels of synaptic proteins, including the SNARE components (Supplemental Figure S1B, C). Consistent with the reduction in PSD95 in males with DS without AD described above, the PT-DS sample showed lower levels of PSD95 (Supplemental Figure S1B, C). To ask if normalizing *APP* gene dose in DS would also normalize the levels of SNARE complexes, we carried out the SNARE complex analysis in the PT-DS frontal cortex. We saw no difference in SNARE complexes in the PT-DS sample versus controls (Supplemental Figure S1D). These results add to the evidence that increased *APP* gene dose is necessary for AD-DS and extend the analysis by indicating that it is also necessary for synapse dysfunction or loss.

3.2.4 Dp16 mice recapitulated reductions in syntaxin 1A and SNAP25 and in SNARE complexes—Studies in mouse models of DS can be used to define common phenotypes and explore underlying pathogenic mechanisms. Studies in mouse models, both the Ts65Dn mouse and the Dp16 mouse, demonstrated similar *App* gene dose dependencies for neuronal loss, tau pathology, changes in endosomes and astrocyte activation [52, 73, 74]. To explore the levels of synaptic proteins in the Dp16 mouse mouse model of DS we examined the same set of synaptic proteins in the cortex of Dp16 mice at age 9-10 months, a time at which changes in endosomal enlargement and astrocyte activation are well established [52]. Only SNARE proteins syntaxin 1A and SNAP25 were significantly reduced (Figure 3A, 3D). Next, to ask if *APP* gene dose impacted synaptic markers we selectively reduced *App* gene copy number in the Dp16 mouse (i.e. Dp16: *App*++−).

Evidence that this effectively reduced *App* gene dose were that APP protein levels were normalized (Figure 3A, 3D). Significantly, in these mice the reductions in both syntaxin 1A and SNAP25 in cortex were prevented (Figure 3A, 3D). It was noteworthy that while the levels of syntaxin 1A and SNAP25 proteins differed by genotype, there were no differences in the levels of their mRNAs, pointing to posttranscriptional events as responsible for the reductions (Supplemental Figure S3).

To determine if the changes in synaptic proteins in Dp16 mice would be reflected in reduced SNARE complex formation, we examined SNARE complexes in Dp16 mice and their age-matched 2N controls at age 9-10 months. As for AD-DS and AD, the SNARE complex was significantly reduced, as revealed in reduced immunostaining for syntaxin 1A at bands of 73 kD, 100-130 kD and also at >180 kD [71] (Figure 2J-2M). Significantly, and consistent with the data for protein levels, the reductions in the Dp16 mouse were dependent on increased *App* gene dose (Figure 2J-2L). Taken together, studies in the Dp16 mice recapitulated the findings in AD-DS for the loss of syntaxin 1A and SNAP25 proteins and the reduction in SNARE complexes. In so doing they also demonstrated a necessary role for increased *APP* gene dose in inducing synaptic changes.

To demonstrate that changes in synaptic proteins were not a developmental phenomenon, we examined both younger Dp16 mice. The levels of syntaxin-1A and SNAP25 were normal in the cortex of 3-4-month-old Dp16 and 2N mice (Figure 3B, 3E). To ask if age exacerbated the changes in synaptic proteins, we examined 20-month-old mice. The changes in Dp16 mice, as compared to controls, again showed reductions only in syntaxin-1A and SNAP25 (Figure 3C, 3F). The reductions appeared to be somewhat larger than those at 9-10 months of age, especially for SNAP25. We conclude that the changes in synaptic proteins in the Dp16 mouse are not developmentally determined but are a function of age. We conclude that age as well as increased *App* gene dose is responsible for changes in synaptic proteins in Dp16 mice, findings consistent with the biology of DS and AD-DS.

3.2.5 The changes of NfL, NPTX2 and syntaxin 1B in the frontal cortex of DS

—Given the importance of synapse function, clinical biomarkers of synapses and axons have been examined in AD and AD-DS [20, 40-44, 46]. Findings of interest have highlighted measures of NfL, NPTX2 and syntaxin 1B. To examine possible relationships between the levels measured in CSF and/or plasma with those in brain we examined the NfL, NPTX2 and syntaxin 1B in the frontal cortex of DS and AD-DS, comparing the values with controls. PMI had no demonstrable effects on the levels of these proteins in AD-DS, DS and their controls (Table 3).

NfL, a neurofilament protein that stabilizes the axonal cytoskeleton is released from axons in normal subjects but in much larger amounts from the axons of those with AD. The increases may reflect breakdown of axons and possibly synapses and thereby serve as marker of disease progression. In AD NfL levels in plasma are correlated with those in CSF; plasma levels were increased to a greater extent in AD than in MCI [75]. Recent studies have also addressed NfL in those with DS, consistently demonstrating age-related increases that exceed levels in controls by the late 30s to 40s [19, 61, 62] – i.e. as long as one to two decades before the average age of dementia diagnosis [62]. We found significantly reduced

NfL levels in AD-DS but not in DS (Figure 4A, 4B). In AD-DS, NfL was significantly decreased in males, with a trend to reductions in females (Supplemental Table 1). However, in DS without AD, while the levels of NfL in females were not reduced, the levels in males were significantly reduced (Supplemental Table 1) pointing to a sex difference in the brain levels of this protein. Interestingly, a sex difference was also noted in controls wherein males had significantly greater levels than females in C/AD-DS group (1.55 ± 0.20 vs. 0.45 ± 0.09; P = 0.0022; Mann-Whitney test) and in C/DS group with the latter not significant. Testing for a possible effect of age we found that age was inversely correlated with NfL levels in AD-DS but not in DS or in their controls (Table 3).

NPTX2, a member of the family of long neuronal pentraxins is secreted from the axons of pyramidal neurons where, after trafficking to excitatory postsynapses, it supports synaptic plasticity [40, 76]. In the AD brain NPTX2 levels were reduced in all cortical regions examined [40]. Reductions in AD brain were correlated with decreased levels of mRNA, possibly due to miR targeting of its mRNA [40]. Recent studies showed decreased CSF NPTX2 in AD relative to controls. NPTX2 levels, as well as the levels of its mRNA were also reduced in the middle frontal gyrus in DS brains, including in subjects as young as 19 years as well as in those diagnosed with AD [40]. Studies in DS [41] found reductions in CSF NPTX2 in all adults with DS, even those without a diagnosis of dementia, but with lower levels corresponding to those diagnosed with dementia. We found that NPTX2 was not reduced in frontal cortex of DS relative to controls (Figure 4A, 4B). In contrast, in AD-DS brains there was a significant reduction in the levels of NPTX2; this change was registered in both females and males (Figure 4A, 4B; Supplemental Table 1). NPTX2 levels did not display a dependence on age within the AD-DS and DS cohorts. (Table 3).

In view of the report that NPTX2 was reported to be decreased in both young DS and AD-DS brains at both the mRNA and protein levels [40] we examined mRNA levels for NPTX2 in frontal cortex in DS and AD-DS subjects (Supplemental Table 5). Compared to controls there was no significant difference in age at death or PMI in DS or AD-DS samples. In contrast to the preserved protein levels in DS, NPTX2 mRNA was reduced. NPTX2 mRNA was further reduced in AD-DS (Supplemental Figure S4).

Syntaxin 1B serves as a SNARE protein. In prior studies, syntaxin 1B was present at control levels in CSF in those with AD [46]. Syntaxin 1B was also examined in CSF in DS and changed little across the continuum from the prodromal to the demented stage [77]. In our studies syntaxin 1B was unchanged relative to controls in DS but was reduced in AD-DS subjects (Figure 4A, 4B). Examining female and male samples separately, trends to lower levels were seen for both females and males (Supplemental Table 1). Syntaxin 1B levels did not vary with age in AD-DS, DS and their controls (Table 3).

To ask if the changes in AD-DS were also found in the Dp16 mouse we measured the levels of NfL and NPTX2 in the Dp16 cortex. At 9-10-month age, there was no difference between 2N and Dp16 in the levels of either protein (Figure 4C, 4D). However, at age 20 months, the levels of NPTX2 were significantly reduced as compared to 2N mice. NfL levels were not reduced even in aged mice (Figure 4C, 4D). These findings point to NPTX2, but not NfL, for detecting changes in the Dp16 mouse, and one that appears later in time than for changes

in SNARE proteins and the synaptic complexes in which they participate. The Dp16 mouse thus recapitulated some but not all the changes in synaptic and axonal measures present in the AD-DS and AD brains.

3.3 DISCUSSION

Reductions in synaptic proteins in AD-DS but not DS: evidence for synaptic failure and loss in the context of dementia and AD neuropathology—Synaptic dysfunction and loss are key features in the pathogenesis of AD [22, 78] and by extension DS in which the pathological manifestations are nearly identical [2, 22], clinical biomarkers are closely related [19-21] and clinical manifestations are consistent with progressive cognitive dysfunction [1, 4, 12, 18]. One approach to understanding the molecular underpinnings of synaptic dysfunction and loss is through examining brain levels of synaptic proteins whose actions support synapse structure and function. Several reports address measures of synaptic proteins in the mild cognitive impairment (MCI) and AD brain [24, 30-38] but little is known of the levels of these proteins in the DS brain before or after diagnosis of AD. Studies in DS offer the opportunity to explore age-related changes in synaptic markers and to ask to what extent they are correlated in time with emerging cognitive deficits.

We examined a number of synaptic proteins in the frontal cortex in DS; samples from those with and without the diagnosis of AD were evaluated. In those diagnosed with AD we found decreased levels of several synaptic proteins, including components of the SNARE complex, synaptic vesicle proteins and PSD95; syntaxin 1A, SNAP25, synaptophysin, synapsin 1 and PSD95 were all reduced (Table 4). That these decreases were significant for synapse function was our finding that SNARE complexes were also reduced in the AD-DS brain. The SNARE complex mediates the fusion of the synaptic vesicle with presynaptic surface membranes to enable neurotransmitter release. Synaptotagmin, the synaptic calcium sensor, showed age-related reductions in the AD-DS cohort, suggesting that this synaptic function is also compromised in the eldest AD-DS patients. Significantly, we failed to detect changes in synaptic proteins or SNARE complexes in those with DS not diagnosed with dementia. These findings are evidence against significant changes in frontal cortex synapses, as measured in synaptic proteins or SNARE complexes, in cognitively stable individuals with DS (Table 4). Remarkably, the changes in the AD-DS brain mirrored those in sporadic AD cases, also examined herein (Table 4). In so doing our findings offer further compelling support that AD-DS and AD share in molecular and cellular events leading to synaptic dysfunction and loss.

The mouse models of DS offer the opportunity to further define the pathogenesis of synaptic change. We asked in the Dp16 mouse model of DS if the changes detected in AD-DS would be recapitulated. Similarities and differences were found. While in mouse model cortex by age 9-10 months we detected reductions in syntaxin 1A and SNAP25, no changes were found for synaptophysin, synapsin 1, or PSD95 (Table 5). The changes in syntaxin 1A and SNAP25 persisted through 20 months of age. Significantly, as for AD-DS and AD, there were significant reductions in SNARE complexes in mouse cortex. We speculate that studies in even older Dp16 mice may reveal changes in the other synaptic proteins reduced in

AD-DS and AD. Importantly, synaptic proteins were not reduced in mice at age 3-4 months, supporting a role for aging in the changes detected. Our findings for reduced synapse function in the mouse model of DS support the utility of studies to elucidate underlying mechanisms in the Dp16 mouse.

Neuronal and synaptic changes during development and with AD-DS—We were surprised to find that synaptic proteins and SNARE complexes were normal in the frontal cortex of those with DS without dementia. Indeed, the essentially universal compromise of intellectual function in children and young adults with DS is correlated with changes in the developing DS brain that include deficits in neurogenesis, neuronal migration, maturation and synaptogenesis [4, 5]. We carefully reviewed the literature to understand how best to interpret our findings on synaptic proteins in the context of developmental changes in the DS brain.

Beginning in utero the brains with those with DS demonstrate reduced weight, reduced overall volume and regional volumes [5]. Development-related changes may contribute to foreshortening of the anterior-posterior dimension, a small cerebellum and failed eversion of the superior temporal gyrus. It has been shown that in frontal cortex there is a 3 to 4-fold reduction in neurogenesis [79] raising the possibility that a number of changes would be detected in frontal cortex throughout life. However, it is not clear what impact developmental changes have on the number of neurons, synapses and dendrites in fetuses, infants, young adults and old adults. In part this is due to the small numbers of DS samples examined as well as the use of different methods by different investigators. As an example, one study at mid-gestation using mass spectrometry showed normal neuron density but reduced SNAP25 [10]. In contrast, at mid-gestation, unbiased stereological methods detected a 35% decrease in cortical cells, counting both neurons and glia [6]. Petit and colleagues, using electron microscopy, found no significant difference in the number of synapses in fetuses at either ~20 or ~40 gestational weeks of age, but found a trend to fewer synapses in DS at the latter time point as well as subtle changes in pre- and post-synapse length [80]. A very recent study used immunostaining and histological methods to examine the DS frontal cortex ranging in age from 28 weeks gestation to 3 years. The authors found reduced cortical thickness, less well-defined cortical lamination, changes in laminar architecture and an apparent delay in neuronal differentiation. However, there was no change in the density of thionin-stained neurons. While there was a significant reduction in calbindin-immunopositive neurons, this represented a small percentage of total neurons [81]. Studies of dendrites have employed the Golgi method to examine dendritic architecture and spine numbers. In visual cortex increases in dendrite branching and length were followed by reductions relative to controls beyond age 6 months and especially after age 2 years [7, 9].

Few studies examined cortical neuron numbers and synapses beyond the postnatal period. Ross and colleagues examined several cortical regions and showed a reduction in the per volume number of small neurons, referred to as granule neurons, of ~60% in a 6 year old girl and 25 year old man. It was noteworthy that there was no consistent pattern of loss for pyramidal neurons in these samples [8]. Cragg, examining the frontal cortex in DS cases

aged 13 years and 27 years, in comparison to control neurosurgical specimens, found no change neuronal density and an increase in synapse counts and synapse density [82].

Neuronal loss in AD-DS mirrors that in AD; in addition to entorhinal cortex, hippocampus and subcortical areas it involves hippocampus, temporal cortex and other cortical areas [17, 57]. In a recent study neuron number was evaluated in the brains of DS subjects at different ages [14]; frontal cortex was not evaluated but entorhinal cortex, hippocampal subfields, subiculum, and several subcortical regions were examined. Young DS subjects (labelled "pre-AD", 26-41 years, 25% with mild dementia) showed region-specific changes relative to young normal controls (25-33 years) that were interpreted as developmentally related. The reductions averaged 28% and involved entorhinal cortex, hippocampal subfields and subiculum. In progressively older cohorts of DS subjects with more prominent cognitive deficits the reductions in neuron number were greater. Synaptic loss in AD-DS is not well documented. However, consistent with the changes in neuron number, the deficits in dendrites and spines present in visual cortex reported in the infantile period appear to be more severe in those with DS diagnosed with dementia [16]. In hippocampus, comparing non-demented DS and AD-DS to controls, the deficit in the number of spines increased from an average of 8% to 17% in CA2-3 and from 17% to 36% in CA1 [15].

Disrupted synapse structure and function underlie onset and progression of cognitive deficits in AD. Early deficits in synapses in AD points to synapse failure as a precursor to neuron loss [23, 24, 30, 83-88]. Studies in AD show that synapse loss precedes neuron loss and that both are closely linked to cognitive dysfunction [87]. The many points of correspondence with AD suggest synapse loss precedes neuronal loss in DS. Supporting this is a recent report that examined the onset of changes in glucose metabolism in DS, an indirect measure of synaptic function, finding that reductions in *fluorodeoxyglucose* (*FDG*)-positron emission tomography (FDG-PET) were evident before reductions in hippocampal volume [89]. Additional studies will be needed to define the relationship between synapse loss and neuronal loss in AD-DS.

The apparent disconnect between reported changes in brain development and our findings on synaptic proteins in the non-demented DS group may be due to several factors. First, is the paucity of studies and data for the DS brain. Indeed, the limited number of samples available for study, the lack of full clinical characterization of these samples, and few recent studies using more advanced quantitative techniques for counting neurons, synapses, and spines combine to limit insights. A second consideration is the even greater paucity of studies examining frontal cortex, the region examined herein. Third, frontal cortex may not most sensitively register degenerative changes involving synapses. This region appears to be impacted later than subcortical regions, entorhinal cortex and certain hippocampal regions [57] and to a lesser extent [14, 17, 58]. Accordingly, our findings distinguishing DS and AD-DS may have shown differences if we had examined entorhinal cortex or hippocampus. Nevertheless, in view of the fact that changes in memory are an early marker of AD-DS [90], any changes in the hippocampus of non-demented DS samples may prove to be largely developmental in origin. Another possibility to explain how a decrease in synapses may not be registered in changes in synaptic proteins would be an increase in the size of synapses interestingly, in prior studies in the Ts65Dn mouse model we discovered an increase in

apposition length in the motor cortex of excitatory synapse [91]. What is clear from our study is that changes in synaptic proteins in frontal cortex are correlated clinically with the changes in attention, executive function and praxis that characterize AD-DS.

Finally, it is possible that changes in cortical function other than synaptic loss may contribute to the differences in cognition noted in children and young adults. Studies in the Ts65Dn mouse model of DS demonstrated increases in the hippocampus in the ratio of inhibitory to excitatory neurotransmission as measured using electrophysiological recordings and through morphological measures showing selective increases in markers of GABAergic synapses [91, 92]. Increased inhibitory neurotransmission has also been demonstrated in the Ts65Dn cortex [93]. Zorilla and colleagues, examining prefrontal cortex in the Ts65Dn mouse, examined the electrophysiology of two inhibitory interneurons: Martinotti cells and parvalbumin-positive neurons. They discovered potentiation of the synaptic inhibitory loop formed between Martinotti cells and pyramidal neurons. In addition, there was increased excitability of parvalbumin interneurons. The result of these changes in inhibition was a significant reduction of pyramidal neuron firing. Taken together with the studies in hippocampus these findings in a mouse model of DS point to over-inhibition due to changes in GABAergic neurotransmission.

The effects of *APP* gene dose and aging on the reduction of synaptic proteins in DS evidence from partial trisomy 21 and Dp16 mice—As a first step in the search for genetic factors that impact synapses in DS, we asked if the increase in *APP* gene dose that is known to be necessary for AD in DS is also responsible for changes in synaptic proteins. People with DS in which partial trisomy creates a genome with only two copies of *APP* have been reported as neither suffering from dementia nor manifesting the neuritic plaques and neurofibrillary tangles of AD [47, 72]. To elucidate a role for increased *APP* gene dose in synaptic loss we examined the frontal cortex of a 72-year-old man with partial trisomy 21 whose genome contained only two copies of *APP* and found that AD-DS-related reductions in synaptic proteins and in the SNARE complex were not recapitulated. In fact, synaptic proteins in the PT-DS sample were comparable to those in younger cognitively normal male controls. Only the level of PSD95 were apparently lower, as in males with DS without AD. The extent to which reduced PSD95 in the frontal cortex of non-demented male DS impacts synaptic function and cognition is unknown. These data support a role for increased *APP* gene dose for synaptic protein reductions in DS. They also point to a non-*APP* gene(s) or other regulatory elements on HSA21 as driving the change of PDS95 in DS.

The necessity of *APP* gene dose to the deficits in synaptic proteins was further supported by studies in the Dp16 mice. While in young mice no changes in synaptic proteins was evident, in older mice reductions in SNAP25 and syntaxin 1A were documented, pointing to an age-related effect on synapse dysfunction and loss. Importantly, increased *App* gene dose was necessary for the changes in syntaxin 1A and SNAP25 (Table 5) and in the SNARE complex. These data complement recent studies of neurodegeneration in the Dp16 mouse demonstrating a role for increased *APP* gene dose [52].

Increased *APP* gene dose is linked to increases in its products in those with DS and AD-DS and in the Dp16 mouse. In the mouse, increases in the full-length protein, its C-terminal fragments and Aβ peptides are evident no later than 4 months of age. An important observation is that in spite of persistent increases in APP and its products in DS and young Dp16 mice the reductions in synaptic proteins occur only in those with AD-DS and in older mice. Several explanations can be envisioned. In one, age itself would impact the levels of *APP* gene dose via changes in the levels of its products, with increasing amounts of a toxic product(s) needed to reach a threshold level to compromise synapses. We noted a 3 to 4-fold increase in the levels of detergent soluble Aβ40 and Aβ38 in AD-DS females compared to DS females; in the Dp16 mouse, there were small but significant age-related increases in the levels of Aβ40 and Aβ42 [52]. Whether changes of this magnitude would suffice to compromise synapses is unknown. The increases in CTFs are consistent with *APP* gene dose in males and but significantly larger in females but females are not more severely affected than males in the reductions in the levels of synaptic proteins in AD-DS. Another possibility is that persisting higher than normal levels of APP products would induce downstream programs whose activities are a function of age, with an impact in only older humans and mice. Thus, it is yet unclear which APP product(s) play an important role in synaptic pathology and how they act to do so.

Insights into the utility of synaptic proteins as biomarkers in DS and AD-DS— Clinical biomarkers play an increasingly important role in defining disease status in those with AD or at risk for AD, with recent efforts also exploring the possibility of predicting the onset of AD in people with DS. To date findings for imaging and fluid biomarkers have demonstrated remarkably similar patterns in AD and AD-DS [19, 62, 94-96]. Our findings recapitulating synaptic protein changes in AD and AD-DS, and partial recapitulation of these changes in the Dp16 mouse, prompted studies of the brain levels of other selected biomarkers now in use to characterize AD progression in DS. In so doing we aimed to ask if changes in biomarkers reflected changes in brain levels.

In MCI and AD NfL levels in plasma are correlated with those in CSF; plasma levels were increased to a greater extent in AD than in MCI. Recent studies have addressed NfL in those with DS, consistently demonstrating age-related increases in NfL in both plasma and CSF that exceed levels in controls by the late 30s to 40s [19, 61, 62]. In those with DS we found that while NfL cortical levels in males as compared to controls were significantly decreased, females with DS did not differ with respect to controls. This suggests that changes in synaptic and axonal structure in males may emerge earlier than in females and be present in the pre-dementia period. Another promising biomarker for AD and AD-DS is NPTX2. In the AD brain NPTX2 levels were reduced in all cortical regions and reductions were correlated with decreased levels of mRNA. The levels of NPTX2 in AD CSF were also significantly decreased [40]. Studies in DS confirm the utility of NPTX2 as a biomarker. Belbin et al., found reductions in CSF NPTX2 in all adults with DS, even those without a diagnosis of dementia, but with lower levels corresponding to those diagnosed with dementia [41]. We found that NPTX2 protein levels were reduced in AD-DS not DS brains; mRNA levels were reduced in both. As yet unexplained is the discrepancy between our finding of normal brain levels in DS and the reduction in CSF levels shown by others [41]. Possibly differences

between DS and AD-DS in mRNA translation, NPTX2 degradation or both could contribute. Interestingly, in the Dp16 mouse NPTX2 levels decreased at older ages than those for reductions in syntaxin 1 and SNAP25 and the SNARE complex (Table 5). This suggests that NPTX2-containing synapses in the model are impacted later than most synapses.

Of special interest are biomarkers that report on the status of synapses and the comparison of fluid biomarker and brain levels. Reductions in frontal cortex in syntaxin 1A and 1B as well as SNAP25 in AD-DS brain raised the possibility that these proteins may prove valuable as biomarkers for DS and AD-DS. Syntaxin 1B levels in DS CSF were not changed across the AD continuum [77]. SNAP25 was demonstrated to be increased in the CSF of those with DS, but only when diagnosed with dementia [21]. In contrast, the maintenance in AD-DS of the levels of synaptobrevin 2, another SNARE protein, suggested it would not prove useful. CSF synaptobrevin 2 levels in those without cognitive decline were significantly lower than for controls. In DS cohorts defined as having prodromal AD or diagnosed with dementia the levels were somewhat higher but with values similar to controls [77]. While for SNAP25 the concordance between decreased cortical and increased CSF levels in AD-DS defines a possible relationship, no simple correlation between CSF and cortical values for the synaptic proteins we discovered to be reduced in AD-DS. It is possible that a broader sampling of brain regions would demonstrate correlations between levels in brain and in CSF and plasma. Thus, additional studies will be needed to demonstrate the utility of biomarkers reporting on synaptic proteins.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

This research was supported by grants from the DH Chen Foundation (R-86U55A, WCM) and NIH R01AG061151, R01AG055523 and R01AG070154 (WCM) and NIH/NIA U19AG068054 (EH). We thank the many participants and their family members for participating in the brain donation programs that made this research possible. We are grateful to NIH NeuroBioBank, UCI MIND ADRC, Banner Sun Health Research Institute and UCSD ADRC for providing frozen human brain tissue specimens. Banner Sun Health Research Institute Brain and Body Donation Program of Sun City, Arizona, for the provision of human biological materials (brain tissue). The Brain and Body Donation Program has been supported by the National Institute of Neurological Disorders and Stroke (U24 NS072026 National Brain and Tissue Resource for Parkinson's Disease and Related Disorders), the National Institute on Aging (P30 AG19610 Arizona Alzheimer's Disease Core Center), the Arizona Department of Health Services (contract 211002, Arizona Alzheimer's Research Center), the Arizona Biomedical Research Commission (contracts 4001, 0011, 05-901 and 1001 to the Arizona Parkinson's Disease Consortium), and the Michael J. Fox Foundation for Parkinson's Research (TB). The tissue provided by the UCI ADRC was supported by NIH/NIA P30AG066519. We thank Drs. Eric Doran and Ira T Lott (University of California Irvine) for caring for the partial trisomy patient and providing the postmortem brain tissue for this case. We thank all the Mobley lab members for fruitful discussions. We also thank Dr. Cassia Overk (University of California San Diego) for helpful comments on the manuscript.

References

[1]. Ballard C, Mobley W, Hardy J, Williams G, Corbett A. Dementia in Down's syndrome. Lancet Neurol. 2016;15:622–36. [PubMed: 27302127]

[2]. Lott IT, Head E. Dementia in Down syndrome: unique insights for Alzheimer disease research. Nat Rev Neurol. 2019;15:135–47. [PubMed: 30733618]

[3]. Bull MJ. Down Syndrome. N Engl J Med. 2020;382:2344–52. [PubMed: 32521135]

[4]. Bartesaghi R, Vicari S, Mobley WC. Prenatal and Postnatal Pharmacotherapy in Down Syndrome: The Search to Prevent or Ameliorate Neurodevelopmental and Neurodegenerative Disorders. Annu Rev Pharmacol Toxicol. 2022;62:211–33. [PubMed: 34990205]

[5]. Stagni F, Giacomini A, Emili M, Guidi S, Bartesaghi R. Neurogenesis impairment: An early developmental defect in Down syndrome. Free Radic Biol Med. 2018;114:15–32. [PubMed: 28756311]

[6]. Larsen KB, Laursen H, Graem N, Samuelsen GB, Bogdanovic N, Pakkenberg B. Reduced cell number in the neocortical part of the human fetal brain in Down syndrome. Ann Anat. 2008;190:421–7. [PubMed: 18722098]

[7]. Becker LE, Armstrong DL, Chan F. Dendritic atrophy in children with Down's syndrome. Ann Neurol. 1986;20:520–6. [PubMed: 2947535]

[8]. Ross MH, Galaburda AM, Kemper TL. Down's syndrome: is there a decreased population of neurons? Neurology. 1984;34:909–16. [PubMed: 6234479]

[9]. Takashima S, Becker LE, Armstrong DL, Chan F. Abnormal neuronal development in the visual cortex of the human fetus and infant with down's syndrome. A quantitative and qualitative Golgi study. Brain Res. 1981;225:1–21. [PubMed: 6457667]

[10]. Weitzdoerfer R, Dierssen M, Fountoulakis M, Lubec G. Fetal life in Down syndrome starts with normal neuronal density but impaired dendritic spines and synaptosomal structure. J Neural Transm Suppl. 2001:59–70. [PubMed: 11771761]

[11]. Esbensen AJ. Health conditions associated with aging and end of life of adults with Down syndrome. Int Rev Res Ment Retard. 2010;39:107–26. [PubMed: 21197120]

[12]. Antonarakis SE, Skotko BG, Rafii MS, Strydom A, Pape SE, Bianchi DW, et al. Down syndrome. Nat Rev Dis Primers. 2020;6:9. [PubMed: 32029743]

[13]. Hithersay R, Startin CM, Hamburg S, Mok KY, Hardy J, Fisher EMC, et al. Association of Dementia With Mortality Among Adults With Down Syndrome Older Than 35 Years. JAMA Neurol. 2019;76:152–60. [PubMed: 30452522]

[14]. Wegiel J, Flory M, Kuchna I, Nowicki K, Wegiel J, Ma SY, 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. [PubMed: 34983655]

[15]. Ferrer I, Gullotta F. Down's syndrome and Alzheimer's disease: dendritic spine counts in the hippocampus. Acta Neuropathol. 1990;79:680–5. [PubMed: 2141748]

[16]. Takashima S, Ieshima A, Nakamura H, Becker LE. Dendrites, dementia and the Down syndrome. Brain Dev. 1989;11:131–3. [PubMed: 2523670]

[17]. Mann DM, Yates PO, Marcyniuk B, Ravindra CR. Loss of neurones from cortical and subcortical areas in Down's syndrome patients at middle age. Quantitative comparisons with younger Down's patients and patients with Alzheimer's disease. J Neurol Sci. 1987;80:79–89. [PubMed: 2956368]

[18]. Silverman W. Down syndrome: cognitive phenotype. Ment Retard Dev Disabil Res Rev. 2007;13:228–36. [PubMed: 17910084]

[19]. Fagan AM, Henson RL, Li Y, Boerwinkle AH, Xiong C, Bateman RJ, et al. Comparison of CSF biomarkers in Down syndrome and autosomal dominant Alzheimer's disease: a cross-sectional study. Lancet Neurol. 2021;20:615–26. [PubMed: 34302786]

[20]. Fortea J, Carmona-Iragui M, Benejam B, Fernandez S, Videla L, Barroeta I, 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;17:860–9. [PubMed: 30172624]

[21]. Henson RL, Doran E, Christian BT, Handen BL, Klunk WE, Lai F, et al. Cerebrospinal fluid biomarkers of Alzheimer's disease in a cohort of adults with Down syndrome. Alzheimers Dement (Amst). 2020;12:e12057. [PubMed: 32671183]

[22]. Chen XQ, Mobley WC. Alzheimer Disease Pathogenesis: Insights From Molecular and Cellular Biology Studies of Oligomeric Abeta and Tau Species. Front Neurosci. 2019;13:659. [PubMed: 31293377]

[23]. DeKosky ST, Scheff SW. Synapse loss in frontal cortex biopsies in Alzheimer's disease: correlation with cognitive severity. Ann Neurol. 1990;27:457–64. [PubMed: 2360787]

[24]. Scheff SW, Price DA, Schmitt FA, DeKosky ST, Mufson EJ. Synaptic alterations in CA1 in mild Alzheimer disease and mild cognitive impairment. Neurology. 2007;68:1501–8. [PubMed: 17470753]

[25]. Martin SB, Dowling AL, Lianekhammy J, Lott IT, Doran E, Murphy MP, et al. Synaptophysin and synaptojanin-1 in Down syndrome are differentially affected by Alzheimer's disease. J Alzheimers Dis. 2014;42:767–75. [PubMed: 24927707]

[26]. Greber S, Lubec G, Cairns N, Fountoulakis M. Decreased levels of synaptosomal associated protein 25 in the brain of patients with Down syndrome and Alzheimer's disease. Electrophoresis. 1999;20:928–34. [PubMed: 10344268]

[27]. Downes EC, Robson J, Grailly E, Abdel-All Z, Xuereb J, Brayne C, et al. Loss of synaptophysin and synaptosomal-associated protein 25-kDa (SNAP-25) in elderly Down syndrome individuals. Neuropathol Appl Neurobiol. 2008;34:12–22. [PubMed: 18005332]

[28]. Sudhof TC. The presynaptic active zone. Neuron. 2012;75:11–25. [PubMed: 22794257]

[29]. Sudhof TC. The synaptic vesicle cycle. Annu Rev Neurosci. 2004;27:509–47. [PubMed: 15217342]

[30]. Scheff SW, Price DA, Schmitt FA, Mufson EJ. Hippocampal synaptic loss in early Alzheimer's disease and mild cognitive impairment. Neurobiol Aging. 2006;27:1372–84. [PubMed: 16289476]

[31]. Masliah E. Mechanisms of synaptic dysfunction in Alzheimer's disease. Histol Histopathol. 1995;10:509–19. [PubMed: 7599445]

[32]. Hamos JE, DeGennaro LJ, Drachman DA. Synaptic loss in Alzheimer's disease and other dementias. Neurology. 1989;39:355–61. [PubMed: 2927643]

[33]. Pham E, Crews L, Ubhi K, Hansen L, Adame A, Cartier A, et al. Progressive accumulation of amyloid-beta oligomers in Alzheimer's disease and in amyloid precursor protein transgenic mice is accompanied by selective alterations in synaptic scaffold proteins. FEBS J. 2010;277:3051–67. [PubMed: 20573181]

[34]. Counts SE, He B, Nadeem M, Wuu J, Scheff SW, Mufson EJ. Hippocampal drebrin loss in mild cognitive impairment. Neurodegener Dis. 2012;10:216–9. [PubMed: 22310934]

[35]. Scheff SW, Price DA, Ansari MA, Roberts KN, Schmitt FA, Ikonomovic MD, et al. Synaptic change in the posterior cingulate gyrus in the progression of Alzheimer's disease. J Alzheimers Dis. 2015;43:1073–90. [PubMed: 25147118]

[36]. Sze CI, Bi H, Kleinschmidt-DeMasters BK, Filley CM, Martin LJ. Selective regional loss of exocytotic presynaptic vesicle proteins in Alzheimer's disease brains. J Neurol Sci. 2000;175:81–90. [PubMed: 10831767]

[37]. Reddy PH, Mani G, Park BS, Jacques J, Murdoch G, Whetsell W Jr., et al. Differential loss of synaptic proteins in Alzheimer's disease: implications for synaptic dysfunction. J Alzheimers Dis. 2005;7:103–17; discussion 73-80. [PubMed: 15851848]

[38]. Leuba G, Savioz A, Vernay A, Carnal B, Kraftsik R, Tardif E, et al. Differential changes in synaptic proteins in the Alzheimer frontal cortex with marked increase in PSD-95 postsynaptic protein. J Alzheimers Dis. 2008;15:139–51. [PubMed: 18780974]

[39]. Tramutola A, Lanzillotta C, Di Domenico F, Head E, Butterfield DA, Perluigi M, et al. Brain insulin resistance triggers early onset Alzheimer disease in Down syndrome. Neurobiol Dis. 2020;137:104772. [PubMed: 31987911]

[40]. Xiao MF, Xu D, Craig MT, Pelkey KA, Chien CC, Shi Y, et al. NPTX2 and cognitive dysfunction in Alzheimer's Disease. Elife. 2017;6.

[41]. Belbin O, Xiao MF, Xu D, Carmona-Iragui M, Pegueroles J, Benejam B, et al. Cerebrospinal fluid profile of NPTX2 supports role of Alzheimer's disease-related inhibitory circuit dysfunction in adults with Down syndrome. Mol Neurodegener. 2020;15:46. [PubMed: 32807227]

[42]. Lewczuk P, Ermann N, Andreasson U, Schultheis C, Podhorna J, Spitzer P, et al. Plasma neurofilament light as a potential biomarker of neurodegeneration in Alzheimer's disease. Alzheimers Res Ther. 2018;10:71. [PubMed: 30055655]

[43]. Zetterberg H, Skillback T, Mattsson N, Trojanowski JQ, Portelius E, Shaw LM, et al. Association of Cerebrospinal Fluid Neurofilament Light Concentration With Alzheimer Disease Progression. JAMA Neurol. 2016;73:60–7. [PubMed: 26524180]

[44]. Ashton NJ, Janelidze S, Al Khleifat A, Leuzy A, van der Ende EL, Karikari TK, et al. A multicentre validation study of the diagnostic value of plasma neurofilament light. Nat Commun. 2021;12:3400. [PubMed: 34099648]

[45]. Bennett MK, Calakos N, Scheller RH. Syntaxin: a synaptic protein implicated in docking of synaptic vesicles at presynaptic active zones. Science. 1992;257:255–9. [PubMed: 1321498]

[46]. Lleo A, Nunez-Llaves R, Alcolea D, Chiva C, Balateu-Panos D, Colom-Cadena M, et al. Changes in Synaptic Proteins Precede Neurodegeneration Markers in Preclinical Alzheimer's Disease Cerebrospinal Fluid. Mol Cell Proteomics. 2019;18:546–60. [PubMed: 30606734]

[47]. Doran E, Keator D, Head E, Phelan MJ, Kim R, Totoiu M, et al. Down Syndrome, Partial Trisomy 21, and Absence of Alzheimer's Disease: The Role of APP. J Alzheimers Dis. 2017;56:459–70. [PubMed: 27983553]

[48]. Pollonini G, Gao V, Rabe A, Palminiello S, Albertini G, Alberini CM. Abnormal expression of synaptic proteins and neurotrophin-3 in the Down syndrome mouse model Ts65Dn. Neuroscience. 2008;156:99–106. [PubMed: 18703118]

[49]. Tramutola A, Lanzillotta C, Barone E, Arena A, Zuliani I, Mosca L, et al. Intranasal rapamycin ameliorates Alzheimer-like cognitive decline in a mouse model of Down syndrome. Transl Neurodegener. 2018;7:28. [PubMed: 30410750]

[50]. Fernandez F, Trinidad JC, Blank M, Feng DD, Burlingame AL, Garner CC. Normal protein composition of synapses in Ts65Dn mice: a mouse model of Down syndrome. J Neurochem. 2009;110:157–69. [PubMed: 19453946]

[51]. Yu T, Li Z, Jia Z, Clapcote SJ, Liu C, Li S, et al. A mouse model of Down syndrome trisomic for all human chromosome 21 syntenic regions. Hum Mol Genet. 2010;19:2780–91. [PubMed: 20442137]

[52]. Sawa M, Overk C, Becker A, Derse D, Albay R, Weldy K, et al. Impact of increased APP gene dose in Down syndrome and the Dp16 mouse model. Alzheimers Dement. 2022;18:1203–34. [PubMed: 34757693]

[53]. DeVos SL, Corjuc BT, Oakley DH, Nobuhara CK, Bannon RN, Chase A, et al. Synaptic Tau Seeding Precedes Tau Pathology in Human Alzheimer's Disease Brain. Front Neurosci. 2018;12:267. [PubMed: 29740275]

[54]. Coomans EM, Schoonhoven DN, Tuncel H, Verfaillie SCJ, Wolters EE, Boellaard R, et al. In vivo tau pathology is associated with synaptic loss and altered synaptic function. Alzheimers Res Ther. 2021;13:35. [PubMed: 33546722]

[55]. Rovelet-Lecrux A, Hannequin D, Raux G, Le Meur N, Laquerriere A, Vital A, et al. APP locus duplication causes autosomal dominant early-onset Alzheimer disease with cerebral amyloid angiopathy. Nat Genet. 2006;38:24–6. [PubMed: 16369530]

[56]. Kwart D, Gregg A, Scheckel C, Murphy EA, Paquet D, Duffield M, et al. A Large Panel of Isogenic APP and PSEN1 Mutant Human iPSC Neurons Reveals Shared Endosomal Abnormalities Mediated by APP beta-CTFs, Not Abeta. Neuron. 2019;104:256–70 e5. [PubMed: 31416668]

[57]. Head E, Lott IT, Wilcock DM, Lemere CA. Aging in Down Syndrome and the Development of Alzheimer's Disease Neuropathology. Curr Alzheimer Res. 2016;13:18–29. [PubMed: 26651341]

[58]. Sadowski M, Wisniewski HM, Tarnawski M, Kozlowski PB, Lach B, Wegiel J. Entorhinal cortex of aged subjects with Down's syndrome shows severe neuronal loss caused by neurofibrillary pathology. Acta Neuropathol. 1999;97:156–64. [PubMed: 9928826]

[59]. Worsdorfer P, I T, Asahina I, Sumita Y, Ergun S. Do not keep it simple: recent advances in the generation of complex organoids. J Neural Transm (Vienna). 2020;127:1569–77. [PubMed: 32385575]

[60]. Dong X, Xu SB, Chen X, Tao M, Tang XY, Fang KH, et al. Human cerebral organoids establish subcortical projections in the mouse brain after transplantation. Mol Psychiatry. 2021;26:2964–76. [PubMed: 33051604]

[61]. Hendrix JA, Airey DC, Britton A, Burke AD, Capone GT, Chavez R, et al. Cross-Sectional Exploration of Plasma Biomarkers of Alzheimer's Disease in Down Syndrome: Early Data from the Longitudinal Investigation for Enhancing Down Syndrome Research (LIFE-DSR) Study. J Clin Med. 2021;10.

[62]. Fortea J, Vilaplana E, Carmona-Iragui M, Benejam B, Videla L, Barroeta I, et al. Clinical and biomarker changes of Alzheimer's disease in adults with Down syndrome: a cross-sectional study. Lancet. 2020;395:1988–97. [PubMed: 32593336]

[63]. Serrano ME, Kim E, Petrinovic MM, Turkheimer F, Cash D. Imaging Synaptic Density: The Next Holy Grail of Neuroscience? Front Neurosci. 2022;16:796129. [PubMed: 35401097]

[64]. Chen XQ, Das U, Park G, Mobley WC. Normal levels of KIF5 but reduced KLC1 levels in both Alzheimer disease and Alzheimer disease in Down syndrome: evidence suggesting defects in anterograde transport. Alzheimers Res Ther. 2021;13:59. [PubMed: 33691783]

[65]. Spandidos A, Wang X, Wang H, Seed B. PrimerBank: a resource of human and mouse PCR primer pairs for gene expression detection and quantification. Nucleic Acids Res. 2010;38:D792–9. [PubMed: 19906719]

[66]. Bartholome O, Van den Ackerveken P, Sanchez Gil J, de la Brassinne Bonardeaux O, Leprince P, Franzen R, et al. Puzzling Out Synaptic Vesicle 2 Family Members Functions. Front Mol Neurosci. 2017;10:148. [PubMed: 28588450]

[67]. Cremona O, Di Paolo G, Wenk MR, Luthi A, Kim WT, Takei K, et al. Essential role of phosphoinositide metabolism in synaptic vesicle recycling. Cell. 1999;99:179–88. [PubMed: 10535736]

[68]. Cremona O, Nimmakayalu M, Haffner C, Bray-Ward P, Ward DC, De Camilli P. Assignment of SYNJ1 to human chromosome 21q22.2 and Synj12 to the murine homologous region on chromosome 16C3-4 by in situ hybridization. Cytogenet Cell Genet. 2000;88:89–90. [PubMed: 10773674]

[69]. Ramakrishnan NA, Drescher MJ, Drescher DG. The SNARE complex in neuronal and sensory cells. Mol Cell Neurosci. 2012;50:58–69. [PubMed: 22498053]

[70]. Tolar LA, Pallanck L. NSF function in neurotransmitter release involves rearrangement of the SNARE complex downstream of synaptic vesicle docking. J Neurosci. 1998;18:10250–6. [PubMed: 9852562]

[71]. Tan GH, Liu YY, Wang L, Li K, Zhang ZQ, Li HF, et al. PRRT2 deficiency induces paroxysmal kinesigenic dyskinesia by regulating synaptic transmission in cerebellum. Cell Res. 2018;28:90–110. [PubMed: 29056747]

[72]. Prasher VP, Farrer MJ, Kessling AM, Fisher EM, West RJ, Barber PC, et al. Molecular mapping of Alzheimer-type dementia in Down's syndrome. Ann Neurol. 1998;43:380–3. [PubMed: 9506555]

[73]. Salehi A, Delcroix JD, Belichenko PV, Zhan K, Wu C, Valletta JS, et al. Increased App expression in a mouse model of Down's syndrome disrupts NGF transport and causes cholinergic neuron degeneration. Neuron. 2006;51:29–42. [PubMed: 16815330]

[74]. Salehi A, Faizi M, Colas D, Valletta J, Laguna J, Takimoto-Kimura R, et al. Restoration of norepinephrine-modulated contextual memory in a mouse model of Down syndrome. Sci Transl Med. 2009;1:7ra17.

[75]. Mattsson N, Andreasson U, Zetterberg H, Blennow K, Alzheimer's Disease Neuroimaging I. Association of Plasma Neurofilament Light With Neurodegeneration in Patients With Alzheimer Disease. JAMA Neurol. 2017;74:557–66. [PubMed: 28346578]

[76]. Chapman G, Shanmugalingam U, Smith PD. The Role of Neuronal Pentraxin 2 (NP2) in Regulating Glutamatergic Signaling and Neuropathology. Front Cell Neurosci. 2019;13:575. [PubMed: 31969807]

[77]. Lleo A, Carmona-Iragui M, Videla L, Fernandez S, Benejam B, Pegueroles J, et al. VAMP-2 is a surrogate cerebrospinal fluid marker of Alzheimer-related cognitive impairment in adults with Down syndrome. Alzheimers Res Ther. 2021;13:119. [PubMed: 34183050]

[78]. Serrano-Pozo A, Frosch MP, Masliah E, Hyman BT. Neuropathological alterations in Alzheimer disease. Cold Spring Harb Perspect Med. 2011;1:a006189. [PubMed: 22229116]

[79]. Lu J, Lian G, Zhou H, Esposito G, Steardo L, Delli-Bovi LC, et al. OLIG2 over-expression impairs proliferation of human Down syndrome neural progenitors. Hum Mol Genet. 2012;21:2330–40. [PubMed: 22343408]

[80]. Petit TL, LeBoutillier JC, Alfano DP, Becker LE. Synaptic development in the human fetus: a morphometric analysis of normal and Down's syndrome neocortex. Exp Neurol. 1984;83:13–23. [PubMed: 6228436]

[81]. Utagawa EC, Moreno DG, Schafernak KT, Arva NC, Malek-Ahmadi MH, Mufson EJ, et al. Neurogenesis and neuronal differentiation in the postnatal frontal cortex in Down syndrome. Acta Neuropathol Commun. 2022;10:86. [PubMed: 35676735]

[82]. Cragg BG. The density of synapses and neurons in normal, mentally defective ageing human brains. Brain. 1975;98:81–90. [PubMed: 123474]

[83]. Ball MJ. Neuronal loss, neurofibrillary tangles and granulovacuolar degeneration in the hippocampus with ageing and dementia. A quantitative study. Acta Neuropathol. 1977;37:111–8. [PubMed: 848276]

[84]. Whitehouse PJ, Price DL, Clark AW, Coyle JT, DeLong MR. Alzheimer disease: evidence for selective loss of cholinergic neurons in the nucleus basalis. Ann Neurol. 1981;10:122–6. [PubMed: 7283399]

[85]. Scheff SW, DeKosky ST, Price DA. Quantitative assessment of cortical synaptic density in Alzheimer's disease. Neurobiol Aging. 1990;11:29–37. [PubMed: 2325814]

[86]. Scheff SW, Price DA. Synapse loss in the temporal lobe in Alzheimer's disease. Ann Neurol. 1993;33:190–9. [PubMed: 8434881]

[87]. Andrade-Moraes CH, Oliveira-Pinto AV, Castro-Fonseca E, da Silva CG, Guimaraes DM, Szczupak D, et al. Cell number changes in Alzheimer's disease relate to dementia, not to plaques and tangles. Brain. 2013;136:3738–52. [PubMed: 24136825]

[88]. Calderon-Garciduenas AL, Duyckaerts C. Alzheimer disease. Handb Clin Neurol. 2017;145:325–37. [PubMed: 28987180]

[89]. Sabbagh MN, Chen K, Rogers J, Fleisher AS, Liebsack C, Bandy D, et al. Florbetapir PET, FDG PET, and MRI in Down syndrome individuals with and without Alzheimer's dementia. Alzheimers Dement. 2015;11:994–1004. [PubMed: 25849033]

[90]. Aschenbrenner AJ, Baksh RA, Benejam B, Beresford-Webb JA, Coppus A, Fortea J, et al. Markers of early changes in cognition across cohorts of adults with Down syndrome at risk of Alzheimer's disease. Alzheimers Dement (Amst). 2021;13:e12184. [PubMed: 33969175]

[91]. Belichenko PV, Kleschevnikov AM, Masliah E, Wu C, Takimoto-Kimura R, Salehi A, et al. Excitatory-inhibitory relationship in the fascia dentata in the Ts65Dn mouse model of Down syndrome. J Comp Neurol. 2009;512:453–66. [PubMed: 19034952]

[92]. Kleschevnikov AM, Belichenko PV, Villar AJ, Epstein CJ, Malenka RC, Mobley WC. Hippocampal long-term potentiation suppressed by increased inhibition in the Ts65Dn mouse, a genetic model of Down syndrome. J Neurosci. 2004;24:8153–60. [PubMed: 15371516]

[93]. Zorrilla de San Martin J, Donato C, Peixoto J, Aguirre A, Choudhary V, De Stasi AM, et al. Alterations of specific cortical GABAergic circuits underlie abnormal network activity in a mouse model of Down syndrome. Elife. 2020;9.

[94]. Abrahamson EE, Head E, Lott IT, Handen BL, Mufson EJ, Christian BT, et al. Neuropathological correlates of amyloid PET imaging in Down syndrome. Dev Neurobiol. 2019;79:750–66. [PubMed: 31379087]

[95]. Nelson LD, Siddarth P, Kepe V, Scheibel KE, Huang SC, Barrio JR, et al. Positron emission tomography of brain beta-amyloid and tau levels in adults with Down syndrome. Arch Neurol. 2011;68:768–74. [PubMed: 21670401]

[96]. Rafii MS, Lukic AS, Andrews RD, Brewer J, Rissman RA, Strother SC, et al. PET Imaging of Tau Pathology and Relationship to Amyloid, Longitudinal MRI, and Cognitive Change in Down Syndrome: Results from the Down Syndrome Biomarker Initiative (DSBI). J Alzheimers Dis. 2017;60:439–50. [PubMed: 28946567]

RESEARCH IN CONTEXT

1. **Systematic Review**: Synaptic protein reductions in Alzheimer's disease (AD) are linked to synapse dysfunction and loss. Although AD is frequent in the elderly with Down syndrome (DS) few studies have examined synaptic proteins in those without or with dementia (AD-DS).
2. **Interpretation**: There were selective reductions in synaptic proteins in frontal cortex in AD-DS brains, but not in DS, or in a case of man with partial trisomy 21 whose genome lacked triplication of the *APP* gene. The changes in AD-DS recapitulated those in AD pointing to shared molecular and cellular events. Studies in the Dp16 model of DS confirmed that *App* gene dose was necessary for reductions in SNARE proteins and showed an age-related dependence for these changes.
3. **Future Directions**: Studies to explore the genetic and molecular events underlying synapse dysfunction and loss in AD and AD-DS are needed to further examine this important feature of pathogenesis. Studies in animal and human DS models could define mechanisms, including the role played by increased *APP* gene dose in AD-DS. Treatments targeting APP or its products may prevent synaptic dysfunction and loss in people with DS.

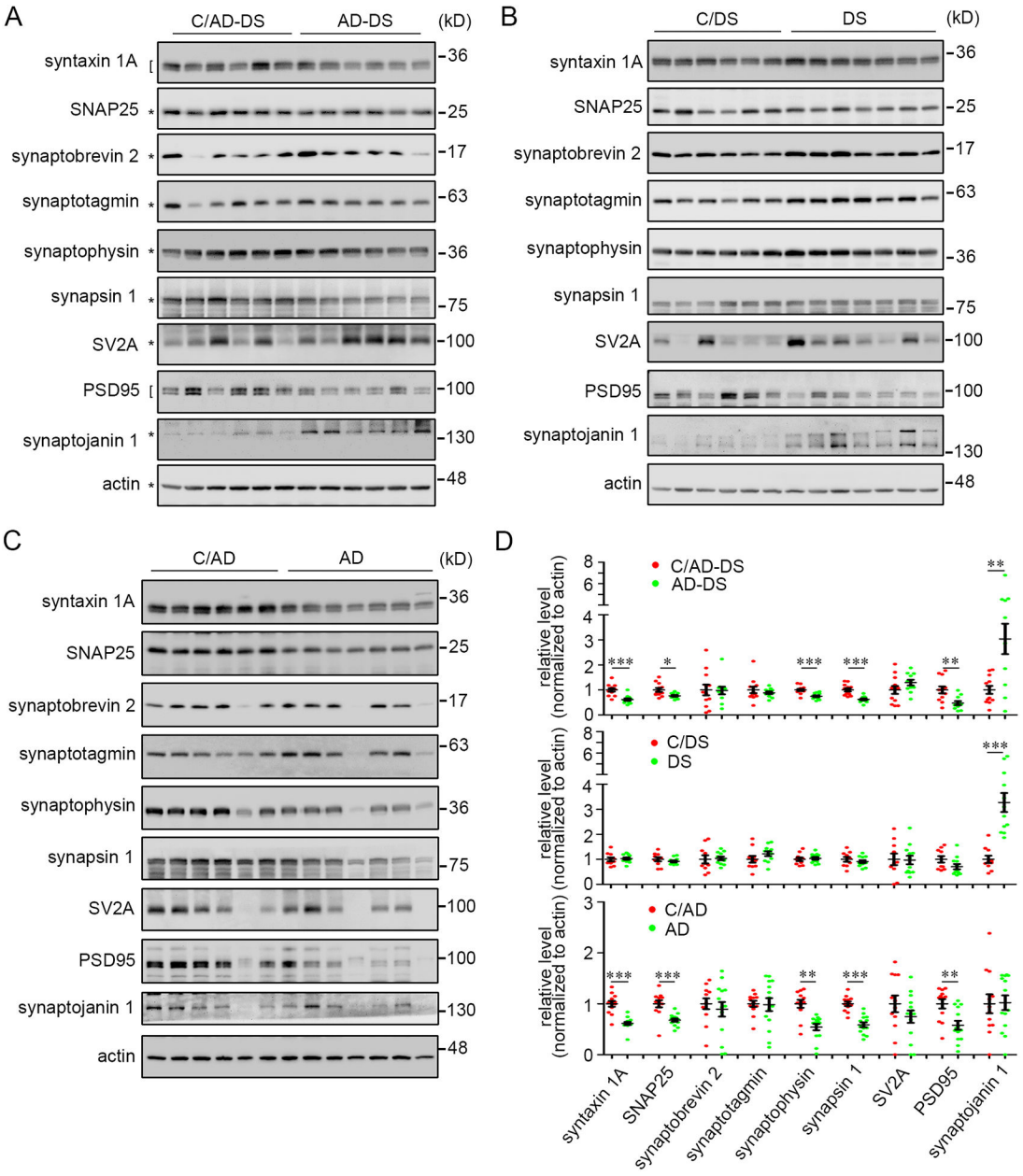

Figure 1.

Selective reduction of synaptic proteins in the frontal cortex of AD-DS and AD but not DS brains. (A-C) Western blotting of synaptic protein levels in protein extracts from the frontal cortex of patients with AD-DS and C/AD-DS (A), DS and C/DS (B), AD and C/AD (C). β-Actin was used as a loading control. (D) Quantitation and statistical analysis of the levels of synaptic proteins from AD-DS and C/AD-DS, DS and C/DS, AD and C/AD samples. Mann-Whitney test; *P < 0.05, **P < 0.01, ***P < 0.001. For all the proteins, the bands marked with (*) and ([) at the right molecular weight were quantitated as stated in the METHODS with the same policy applied to the bands for the same proteins from other samples.

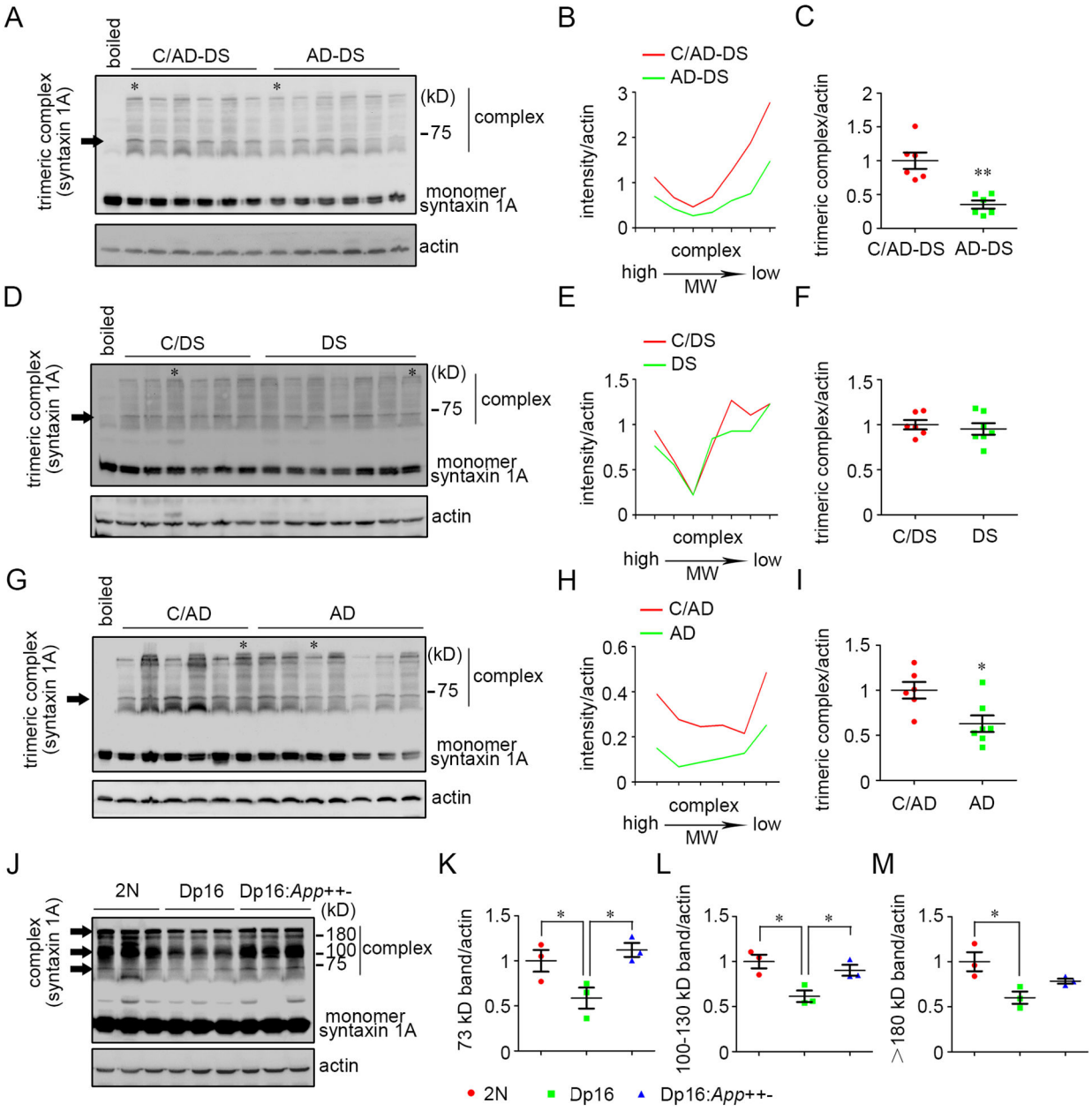

Figure 2.

Reduction of SNARE complexes in AD-DS, AD and Dp16 samples. Syntaxin 1A was present in a 35 kD monomeric form and in a 73 kD trimeric complex with SNAP25 and synaptobrevin 2 as well as other high molecular weight forms. (A, D, G) Unboiled AD-DS (A), DS (D) and AD (G) SDS samples were loaded on SDS-PAGE and probed with the syntaxin 1A antibody. A boiled sample was also loaded as a control to demonstrate disruption of the complexes with heating. The arrow points to the 73 kD trimeric band. (B, E, H) Profiles of relative intensities of the ladder of complexes from the representative samples marked with asterisks; the heaviest complexes are represented at the left end of the x-axis. (C, F, I) Quantitation and statistical analysis of the levels of 73 kD trimeric SNARE complex in AD-DS and DS samples. Mann-Whitney test; *P < 0.05, **P < 0.01.

(J) Unboiled 2N, Dp16 and Dp16: *App*++− samples from mice aged 9 to 10 months were loaded on SDS-PAGE and probed with the syntaxin 1A antibody. Arrows point to the major 73 kD, 100-130 kD and >180 kD bands. (K-M) Quantitation and statistical analysis of the levels of 73 kD, 100-130 kD and >180 kD bands. One-way ANOVA test followed by Newman-Keuls Multiple Comparison Test. n = 3 mice for each category; *P < 0.05.

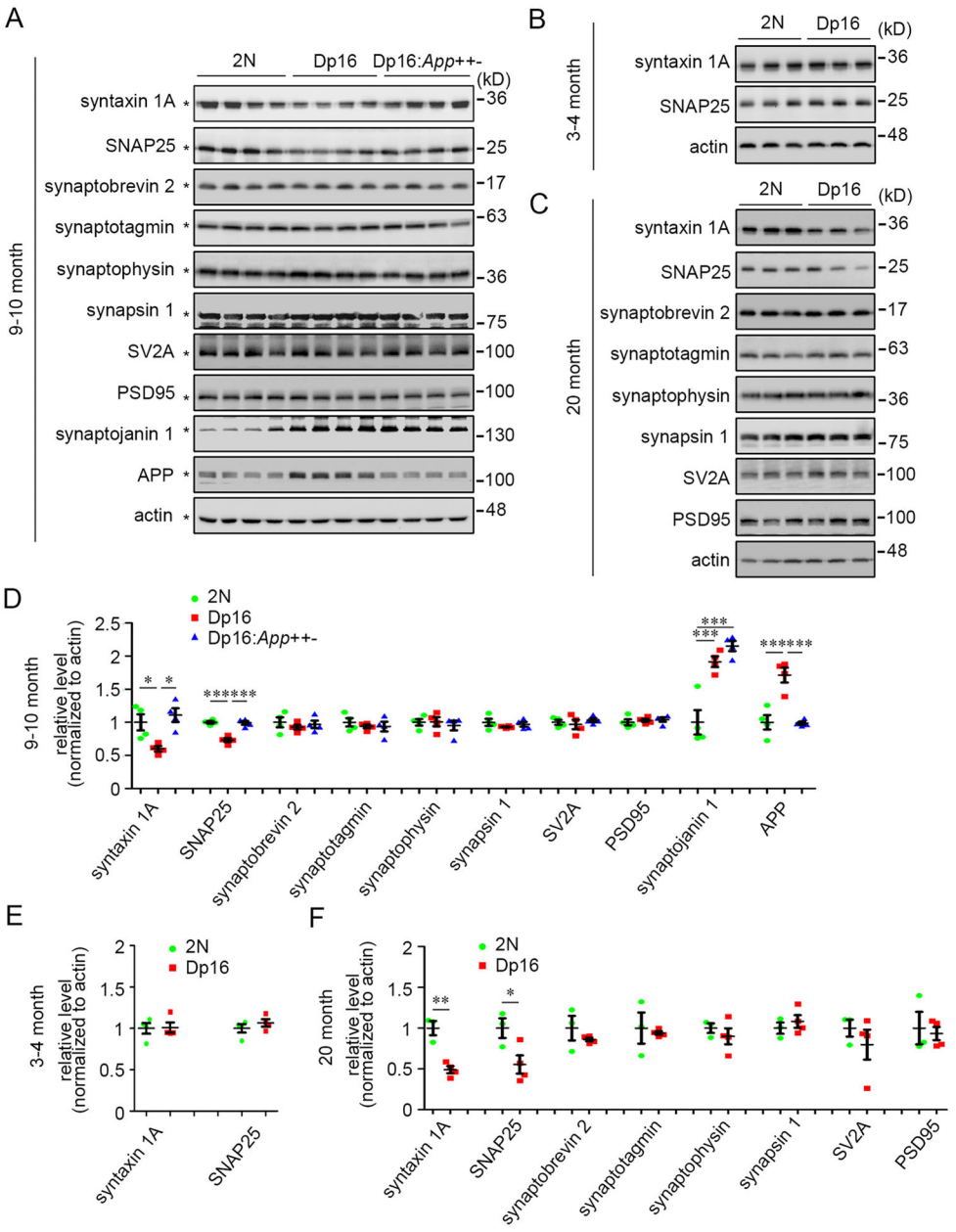

Figure 3.

Selective change of synaptic proteins in the cortex of Dp16 mice. (A) Western blotting analysis of the levels of synaptic proteins in the cortex from 9-10-month-old Dp16 mice. fl-mAPP (full-length mouse APP) was also probed. (B, C) Western blotting analysis of the levels of synaptic proteins in the cortex from 3-4-month-old (B) and 20-month-old (C) Dp16 mice. β-actin was used as a loading control. (D-F) Quantitation and statistical analysis of the levels of synaptic proteins and APP in Dp16 with three copies of *App* gene or with two copies (Dp16: *App*++−) mice. One-way ANOVA test followed by Newman-Keuls Multiple Comparison Test and n = 4 mice for each category for D; unpaired student *t*-test. n = 3-4 mice for each category for E, F. *P < 0.05, **P < 0.01, ***P < 0.001. For all the proteins, the bands marked with (*) at the right molecular weight were quantitated as stated in the

METHODS with the same policy applied to the bands for the same proteins from other samples.

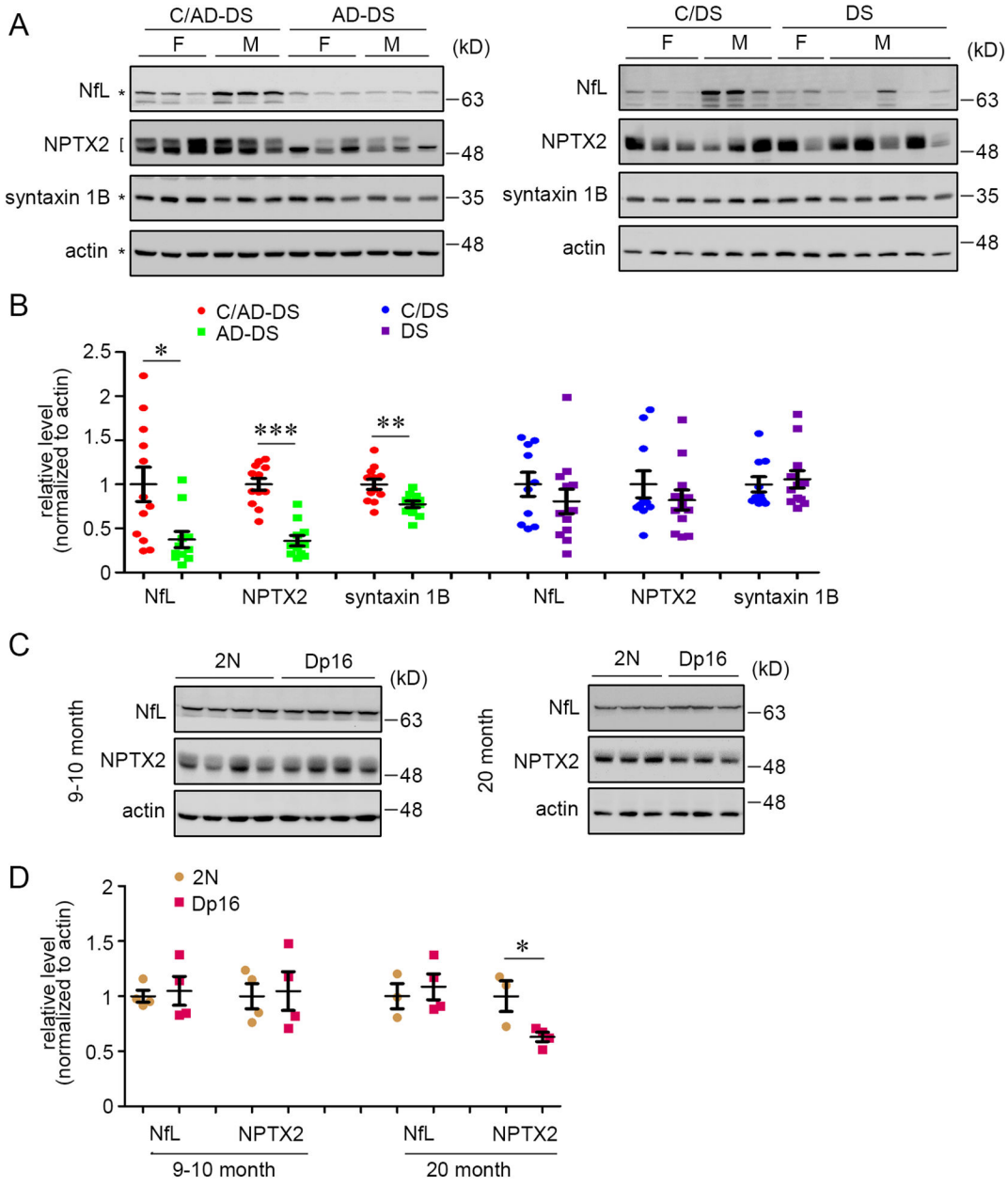

Figure 4.

Levels of selective biomarkers in the frontal cortex of AD-DS, DS and the cortex of Dp16 mice. (A) Western blotting analysis of the levels of NfL, NPTX2 and syntaxin1B in the frontal cortex of patients with DS, AD-DS, C/DS or C/AD-DS. β-actin was used as a loading control. (B) Quantitation and statistical analysis of the levels of NfL, NPTX2 and syntaxin 1B in females and males samples combined. Mann-Whitney test; *P < 0.05, **P < 0.01, ***P < 0.001. (C) Western blotting analysis of the levels of NfL and NPTX2 in the cortex from 9-10-month-old and 20-month-old Dp16 mice. β-actin was used as a loading control. (D) Quantitation and statistical analysis of the levels of NfL and NPTX2 in Dp16 mice. Unpaired student *t*-test; n = 4 mice for each category for 9-10-month-old mice, n = 3-4 mice for each category for 20-month-old mice; *P < 0.05. For all the proteins, the

bands marked with (*) and ([) at the right molecular weight were quantitated as stated in the METHODS with the same policy applied to the bands for the same proteins from other samples.

Table 1.

Demographics, PMI, and clinical diagnosis for AD-DS, C/AD-DS, DS and C/DS frontal cortex samples

Diagnosis	Gender	Age (Years)	Post-mortem interval (h)	Diagnosis	Neuropathological diagnosis
C/AD-DS 1	Female	49	7	Cognitively normal	
C/AD-DS 2	Female	50	7	Cognitively normal	
C/AD-DS 3	Female	46	18	Cognitively normal	
C/AD-DS 4	Male	48	6	Cognitively normal	
C/AD-DS 5	Male	57	16	Cognitively normal	
C/AD-DS 6	Male	58	19	Cognitively normal	
C/AD-DS 7	Female	54	6	Cognitively normal	
C/AD-DS 8	Female	39	19	Cognitively normal	
C/AD-DS 9	Female	59	5.9	Cognitively normal	
C/AD-DS 10	Male	59	5.9	Cognitively normal	
C/AD-DS 11	Male	59	15.8	Cognitively normal	
C/AD-DS 12	Male	58	9	Cognitively normal	
Mean ± SEM	6F/6M	53 ± 2	11.2 ± 1.7		
AD-DS 1	Female	57	6	Trisomy 21 with AD	Brain atrophy; classic, extensive amyloid deposition and neurofibrillary degeneration
AD-DS 2	Female	51	4	Trisomy 21 with AD	Senile cerebral disease, Alzheimer's type; congophilic angiopathy
AD-DS 3	Female	46	7	Trisomy 21 with AD	Diffuse and severe
AD-DS 4	Male	53	23	Trisomy 21 with AD	AD neuropathological changes and cerebral amyloid angiopathy
AD-DS 5	Male	55	12	Trisomy 21 with AD	AD neuropathological changes and cerebral amyloid angiopathy
AD-DS 6	Male	57	5	Trisomy 21 with AD	Advanced AD
AD-DS 7	Female	55	25	Trisomy 21 with AD	n/a
AD-DS 8	Male	65	10	Trisomy 21 with AD	Diffuse and severe
AD-DS 9	Male	41	15	Trisomy 21 with AD	Advanced
AD-DS 10	Male	56	16	Trisomy 21 with AD	Advanced
AD-DS 11	Male	64	20	Trisomy 21 with AD	MMSE 21/30 9 years before death
Mean ± SEM	4F/7M	55 ± 2	13.0 ± 2.2		
C/DS 1	Female	48	8	Cognitively normal	
C/DS 2	Female	51	21	Cognitively normal	
C/DS 3	Female	57	7	Cognitively normal	
C/DS 4	Male	52	4	Cognitively normal	
C/DS 5	Male	51	17	Cognitively normal	
C/DS 6	Male	55	22	Cognitively normal	

Diagnosis	Gender	Age (Years)	Post-mortem interval (h)	Diagnosis	Neuropathological diagnosis
C/DS 7*	Female	50	7	Cognitively normal	
C/DS 8*	Female	46	18	Cognitively normal	
C/DS 9	Female	51	8	Cognitively normal	
C/DS 10	Male	57	6.6	Cognitively normal	
Mean ± SEM	6F/4M	52 ± 1	11.9 ± 2.2		
DS 1	Female	50	23	Trisomy 21 without AD	n/a
DS 2	Female	39	12	Trisomy 21 without AD	n/a
DS 3	Male	22	15	Trisomy 21 without AD	n/a
DS 4	Male	40	10	Trisomy 21 without AD	Diffuse beta-amyloid deposition in the neocortex
DS 5	Male	25	22	Trisomy 21 without AD	Rare neocortical disffuse plaques
DS 6	Male	25	24	Trisomy 21 without AD	n/a
DS 7	Male	57	22	Trisomy 21 without AD	Frequent neuritic plaques, no significant neurofibrillary pathology
DS 8	Female	42	5	Trisomy 21 without AD	Braak stage V
DS 9	Female	48	18.4	Trisomy 21 without AD	Braak stage III
DS 10	Female	51	2.7	Trisomy 21 without AD	Braak stage III
DS 11	Female	62	7	Trisomy 21 without AD	Braak stage III
DS 12	Male	23	24	Trisomy 21 without AD	n/a
Mean ± SEM	6F/6M	40 ± 4 (P=0.0340 vs C/DS)	15.4 ± 2.3		
	6F/2M	49 ± 3¶	12.5 ± 2.8¶		

*control samples used for both DS and for AD-DS

¶analysis excluding the four young DS samples (DS 3, DS 5, DS 6 and DS 12). n/a, not available

Table 2.

Spearman's correlation analysis between synaptic proteins and age in human control, AD-DS, DS and AD frontal cortex samples.

Diagnosis: synaptic protein	Parameter	Sample size (n)	Correlation coefficient (r)	Significance (P)
AD-DS: synaptobrevin 2	Age	11	−0.3470	0.2957
C/AD-DS: synaptobrevin 2	Age	12	0.2363	0.4596
AD-DS: SV2A	Age	11	−0.4201	0.1983
C/AD-DS: SV2A	Age	12	−0.1693	0.5988
AD-DS: synaptotagmin	Age	11	−0.6256	0.0395*
C/AD-DS: synaptotagmin	Age	12	−0.05291	0.8703
AD-DS: synaptophysin	Age	11	−0.4429	0.1725
C/AD-DS: synaptophysin	Age	12	0.2787	0.3804
AD-DS: synapsin 1	Age	11	−0.05023	0.8834
C/AD-DS: synapsin 1	Age	12	0.04586	0.8875
AD-DS: syntaxin 1A	Age	11	0.1050	0.7586
C/AD-DS: syntaxin 1A	Age	12	0.02469	0.9393
AD-DS: PSD95	Age	11	0.2009	0.5536
C/AD-DS: PSD95	Age	12	0.2046	0.5236
AD-DS: SNAP25	Age	11	−0.6347	0.0359*
C/AD-DS: SNAP25	Age	12	−0.3845	0.2172
AD-DS: synaptojanin 1	Age	11	0.1279	0.7079
C/AD-DS: synaptojanin 1	Age	12	0.2787	0.3804
DS: synaptobrevin 2	Age	12	−0.4834	0.1114
C/DS: synaptobrevin 2	Age	10	0.3323	0.3481
DS: SV2A	Age	12	−0.1576	0.6247
C/DS: SV2A	Age	10	0.03693	0.9193
DS: synaptotagmin	Age	12	−0.4553	0.1369
C/DS: synaptotagmin	Age	10	0.2708	0.4492
DS: synaptophysin	Age	12	−0.3958	0.2028
C/DS: synaptophysin	Age	10	−0.2400	0.5042
DS: synapsin 1	Age	12	−0.4168	0.1777
C/DS: synapsin 1	Age	10	−0.2339	0.5155
DS: syntaxin 1A	Age	12	−0.01401	0.9655
C/DS: syntaxin 1A	Age	10	−0.1846	0.6096
DS: PSD95	Age	12	−0.1856	0.5635
C/DS: PSD95	Age	10	0.1600	0.6588
DS: SNAP25	Age	12	0.04904	0.8797
C/DS: SNAP25	Age	10	−0.2400	0.5042

Diagnosis: synaptic protein	Parameter	Sample size (n)	Correlation coefficient (r)	Significance (P)
DS: synaptojanin 1	Age	12	−0.1751	0.5862
C/DS: synaptojanin 1	Age	10	0.5293	0.1157
AD: synaptobrevin 2	Age	14	0.05947	0.8400
C/AD: synaptobrevin 2	Age	12	0.05975	0.8536
AD: SV2A	Age	14	0.1235	0.6741
C/AD: SV2A	Age	12	−0.02812	0.9309
AD: synaptotagmin	Age	14	0.1696	0.5621
C/AD: synaptotagmin	Age	12	−0.2144	0.5034
AD: synaptophysin	Age	14	−0.3789	0.1816
C/AD: synaptophysin	Age	12	−0.1371	0.6710
AD: synapsin 1	Age	14	−0.2115	0.4680
C/AD: synapsin 1	Age	12	0.1019	0.7526
AD: syntaxin 1A	Age	14	0.2511	0.3865
C/AD: syntaxin 1A	Age	12	−0.1968	0.5398
AD: PSD95	Age	14	−0.06388	0.8283
C/AD: PSD95	Age	12	−0.2320	0.4681
AD: SNAP25	Age	14	−0.09031	0.7588
C/AD: SNAP25	Age	12	−0.4007	0.1968
AD: synaptojanin 1	Age	14	−0.01101	0.9702
C/AD: synaptojanin 1	Age	12	−0.3726	0.2330

Table 3.

Spearman's correlation analysis between selective biomarkers and age or PMI in human AD-DS and control frontal cortex samples.

Diagnosis: biomarker	Parameter	Sample size (n)	Correlation coefficient (r)	Significance (P)
AD-DS: NfL	Age	11	0.7443	0.0086**
AD-DS: NPTX2	Age	11	−0.1370	0.6879
AD-DS: syntaxin 1B	Age	11	0.3744	0.2566
C/AD-DS: NfL	Age	12	0.5468	0.0658
C/AD-DS: NPTX2	Age	12	−0.1094	0.7351
C/AD-DS: syntaxin 1B	Age	12	−0.08819	0.7852
DS: NfL	Age	12	0.2732	0.3902
DS: NPTX2	Age	12	−0.4799	0.1144
DS: syntaxin 1B	Age	12	0.5464	0.0660
C/DS: NfL	Age	10	0.3200	0.3673
C/DS: NPTX2	Age	10	−0.4554	0.1859
C/DS: syntaxin 1B	Age	10	0.5847	0.0759
AD-DS: NfL	PMI	11	−0.3000	0.3701
AD-DS: NPTX2	PMI	11	−0.1455	0.6696
AD-DS: syntaxin 1B	PMI	11	−0.1818	0.5926
C/AD-DS: NfL	PMI	12	−0.2359	0.4604
C/AD-DS: NPTX2	PMI	12	0.01408	0.9653
C/AD-DS: syntaxin 1B	PMI	12	0.2007	0.5317
DS: NfL	PMI	12	−0.3158	0.3173
DS: NPTX 2	PMI	12	0.5053	0.0938
DS: syntaxin 1B	PMI	12	−0.2316	0.4689
C/DS: NfL	PMI	10	−0.5854	0.0754
C/DS: NPTX2	PMI	10	0.5915	0.0717
C/DS: syntaxin 1B	PMI	10	−0.2378	0.5082

Table 4.

Summary of the levels of synaptic proteins and NfL in the frontal cortex of humans.

	DS vs control	AD-DS vs control	AD vs control
syntaxin 1A	→	↓	↓
SNAP25	→	↓	↓
synaptobrevin 2	→	→	→
synaptotagmin	→	→	→
synaptophysin	→	↓	↓
synapsin 1	→	↓	↓
SV2A	→	→	→
PSD95	→	↓	↓
synaptojanin 1	↑	↑	→
NfL	→	↓	n/a
NPTX2	→	↓	n/a
syntaxin 1B	→	↓	n/a

n/a, not available

Table 5.

Summary of the levels of synaptic proteins and NfL in the cortex of Dp16 mouse model.

	Young Dp16 vs ctrl (3-4 m)	**Mid-age Dp16 vs 2N (9-10 m)**	**Mid-age Dp16: *App*++- vs 2N (9-10 m)**	**Old Dp16 vs 2N (20 m)**
syntaxin 1A	→	↓	→	↓
SNAP25	→	↓	→	↓
synaptobrevin 2	n/a	→	→	→
synaptotagmin	n/a	→	→	→
synaptophysin	n/a	→	→	→
synapsin 1	n/a	→	→	→
SV2A	n/a	→	→	→
PSD95	n/a	→	→	→
synaptojanin 1	n/a	↑	↑	n/a
NfL	n/a	→	n/a	→
NPTX2	n/a	→	n/a	↓
syntaxin 1B	n/a	n/a	n/a	n/a

n/a, not available