Title

An investigation of the molecular mechanisms engaged before and after the development of Alzheimer disease neuropathology in Down syndrome: a proteomics approach

Authors

Cenini, Giovanna
Fiorini, Ada
Sultana, Rukhsana
et al.

Publication Date

2014-11-01

DOI

10.1016/j.freeradbiomed.2014.08.006

Peer reviewed

Published in final edited form as:
Free Radic Biol Med. 2014 November ; 0: 89–95. doi:10.1016/j.freeradbiomed.2014.08.006.

An Investigation of the Molecular Mechanisms Engaged Prior and Subsequent to the Development of Alzheimer Disease Neuropathology in Down Syndrome: A Proteomics Approach

Giovanna Cenini[1,°], **Ada Fiorini**[1,2,°], **Rukhsana Sultana**[1], **Marzia Perluigi**[2], **Jian Cai**[3], **Jon B. Klein**[3], **Elizabeth Head**[4], and **D. Allan Butterfield**[1,*]

[1]Department of Chemistry, Center of Membrane Sciences, Sanders Brown Center on Aging, University of Kentucky, Lexington, KY 40506 USA

[2]Department of Biochemical Sciences, Sapienza University of Rome, 00185 Rome, Italy

[3]Division of Nephrology, Department of Medicine and Proteomics Center, University of Louisville, Louisville, KY 40292, USA

[4]Department of Molecular and Biomedical Pharmacology, and Sanders-Brown Center on Aging, University of Kentucky, Lexington, KY, USA 40536

Abstract

Down syndrome (DS) is one of the most common causes of intellectual disability, due to trisomy of all or part of chromosome 21. DS is also associated with the development of Alzheimer disease (AD) neuropathology after the age of 40 years. To better clarify the cellular and metabolic pathways that could contribute to the differences in DS brain, in particular those involved in the onset of neurodegeneration, we analyzed the frontal cortex of DS subjects with or without significant AD pathology in comparison with age-matched controls, using a proteomics approach. Proteomics represents an advantageous tool to investigate the molecular mechanisms underlying the disease. From these analyses, we investigated the effects that age, DS, and AD neuropathology could have on protein expression levels. Our results show overlapping and independent molecular pathways (including energy metabolism, oxidative damage, protein synthesis and autophagy) contributing to DS, to aging and to the presence of AD pathology in DS. Investigation of pathomechanisms involved in DS with AD, may provide putative targets for therapeutic approaches to slow the development of AD.

Keywords

Down syndrome; Trisomy 21; proteomics; neuropathology; Alzheimer disease

[*]**Corresponding author:** Prof. D. Allan Butterfield, Department of Chemistry, Center of Membrane Sciences, and Sanders-Brown, Center on Aging, University of Kentucky, Lexington, KY, USA 40536, dabcns@uky.edu.
[°]Authors contributed equally

Introduction

Down syndrome (DS) or trisomy 21 is the most frequent genetic disorder most commonly caused by triplication of chromosome 21 [1]. DS is associated with developmental abnormalities of the central nervous system that leads to intellectual disability [2]. In addition to intellectual disability, virtually all DS brains over 40 years of age show Alzheimer disease (AD) neuropathology [3], including the presence of senile plaque (SP) and neurofibrillary tangles (NFT) [3–5], and clinical signs of dementia become more frequent after 50 years of age [6, 7]. Several studies support the hypothesis that the over-expression of genes present on chromosome 21 is responsible for the features of DS, including the development of AD [8, 9]. The over-expression of amyloid precursor protein (APP), due to its location on chromosome 21, leads to an enhanced production of beta-amyloid (Aβ), the principle component of SP. Mutations in the APP gene are related to familial AD [10], and therefore APP over-expression and subsequent Aβ formation may be a crucial event leading to the development of AD pathology in DS [11, 12]. Aβ deposition in DS brains begins in the early years of the lifespan, as young as eight years, and increases progressively with increasing age [13, 14].

Further, oxidative damage appears to have a key role in DS and in the exacerbation of AD [15–17]. Among many genes located on chromosome 21 linked with oxidative damage and ROS production, superoxide dismutase (SOD-1) is the most relevant, since its up-regulation without a similar increase of catalase leads to an accumulation of hydrogen peroxide [12, 17], and consequently to increased oxidative stress levels. In addition, enhanced release of Aβ, both Aβ (1–40/42), also could contribute to oxidative damage [13, 18]. As noted above, neuropathological features of dementia are manifested at a younger age in people with DS relative to the general population, but it appears delayed relative to AD neuropathology, suggesting the presence of compensatory mechanisms [19, 20]. Therefore, a better understanding of these compensatory responses and an eventually manipulation of these mechanisms may be therapeutically beneficial for people with DS. Thus, individuals with DS could provide an understanding of the early alterations leading to AD, and therefore to AD itself.

To clarify the cellular and metabolic pathways that could contribute to the pathomechanisms of the DS brain, in particular those responsible for the onset of AD neuropathology, we analyzed the frontal cortex of DS autopsy cases with or without AD pathology in comparison with age-matched non-DS controls, using a proteomics approach. Several studies from the past ten years have already shown many proteins from different pathways to be dysregulated in DS fetal brains [21, 22].

The aim of our study was to identify the proteins and associated pathways compromised in DS as compared to people without DS to determine which pathways are different as a function of trisomy 21. Second, a comparison of younger individuals with DS and without AD to older DS cases with AD neuropathology was used to determine if specific pathways distinguish the two groups. Establishing novel cellular mechanisms and pathways that

appear to contribute to the DS phenotype and additional development of AD neuropathology may provide putative novel targets for therapeutic intervention.

Materials and methods

Subjects

DS and young or non-demented older control cases were obtained from the University of California-Irvine-Alzheimer Disease Research Center Brain Tissue Repository, the Eunice Kennedy Shriver NICHD Brain and Tissue Bank for Developmental Disorders, and the University of Kentucky Alzheimer Disease Center. Table 1 shows the characteristics of the cases used. DS cases were divided into two groups, with or without sufficient pathology for a neuropathologic diagnosis of AD. All cases with both DS and AD, referred to as DSAD, were over the age of 40 years. Thus for the current study, controls were split into two groups, either less than or equal to 40 years or older than 40 years at death. The post mortem interval (PMI) was different across groups, with the DSAD group overall having a lower PMI ($F(3,66)=7.30$ $p< 0.0005$). A subset of these autopsy cases was used in previous experiments measuring insoluble Aβ as a function of age in DS [18], in a study of redox proteomics [23], and oxidative damage [24].

Sample preparation

Samples from the frontal cortex of non-DS controls, DS, and DSAD were thawed in lysis buffer (pH 7.4) containing 320 mM sucrose, 1% of 1.0 M Tris–HCl (pH=8.8), 0.098 mM $MgCl_2$, 0.076 mM EDTA, proteinase inhibitors leupeptin (0.5 mg/ml), pepstatin (0.7 μg/ml), aprotinin (0.5 mg/ml), and phosphatase inhibitor cocktail (Sigma Aldrich, St. Louis, MO). The brains were homogenized by 20 passes of Wheaton tissue homogenizer, and the resulting homogenate was centrifuged at 14,000g for 10 min to remove cellular debris. The supernatant was extracted to determine the total protein concentration by the BCA method (Pierce,Rockford, IL).

Two-dimensional electrophoresis

Proteins (150 μg) were precipitated in 15% final concentration of trichloroacetic acid for 10 min in ice. Each individual sample (six per group) was then spun down at 10 000 g for 5 min and precipitates were washed in ice-cold ethanol-ethyl acetate (1:1) solution four times. The final pellet was dissolved in 200 μL of 8 M urea, 2% CHAPS, 2 M thiourea, 20 mM dithiothreitol, 0.2% of ampholytes (Bio-Rad, Hercules, CA, USA) and bromophenol blue, and placed in agitation for 3 hours. Solubilized proteins were then sonicated twice for 30 sec. Samples were loaded on 110-mm pH 3–10 immobilized pH gradients strips in a Bio-Rad Isoelectric focusing Cell system (Bio-Rad). Following 18 h of active rehydration (50 V), isoelectric focusing was performed as previously reported [25]. The focused isoelectric focusing strips were stored at −80°C until a second dimension electrophoresis was performed. For the second dimension, thawed strips were sequentially equilibrated for 15 min in the dark in 375 mM Tris pH 8.8, 6 M urea, 2% sodiumdodecyl sulfate, 20% glycerol containing first 2% dithiothreitol, and then 2.5% of iodoacetamide. Sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis was performed in Criterion Tris-HCl Gels 8–16% (Bio-Rad) at 200 V for 1 h.

Gel staining and image analysis

Gels were fixed for 45 min in 10% methanol, 7% acetic acid and stained overnight with SYPRO Ruby gel stain (Bio-Rad). After destaining in deionized water, gels were scanned with a STORM UV transilluminator (kex = 470 nm, kem = 618 nm; Molecular Dynamics, Sunnyvale, CA, USA). Images obtained were saved in Tagged Image File Format Gel and imaging was software-aided using PD-Quest (Bio-Rad) imaging software. Briefly, a master gel was selected followed by normalization of all gels according to the total spot density. Then, a manual matching of common spots that could be visualized among the differential 2D gels was performed. After obtaining a significant number of spots, the automated matching of all spots was then initiated. Automated matching is based on user-defined parameters for spot detection. These parameters are based on the faint test spot, the largest spot, and the largest spot cluster that occur in the master gel and are defined by the user. This process generates a large pool of data, ~350 spots. Only proteins showing computer determined significant differential levels between the two groups being analyzed were considered for identification. To determine significant differential levels of proteins, analysis sets were created using the analysis set manager software incorporated into the PD-Quest software. The numbers of pixels that occur in a protein spot, corresponding to an increase/decrease in protein level, were computed by the software. A quantitative analysis set was created that recognized matched spots with differences in the number of pixels that occur in each spot. Then, a statistical analysis set was created that recognized matched spots with differences in the number of pixels that occur in each spot and a statistical analysis set was created that used a Student's t-test at 95% confidence to identify spots with p-values < 0.05. Spots with $p < 0.05$ were considered significant.

In-gel trypsin digestion

Briefly, protein spots identified as significantly altered after PD-Quest analysis were excised from 2D-gels with a clean, sterilized blade and transferred to Eppendorf microcentrifuge tubes. Gel plugs were then washed with 0.1 M ammonium bicarbonate (NH_4HCO_3) at room temperature for 15 min, followed by incubation with 100% acetonitrile at room temperature for 15 min. After solvent removal, gel plugs were dried in their respective tubes under a flow hood at room temperature (RT). Plugs were incubated for 45 min in 20 μl of 20 mM DTT in 0.1 M NH_4HCO_3 at 56 °C. The DTT/NH_4HCO_3 solution was then removed and replaced with 20 μl of 55 mM IA in 0.1 M NH_4HCO_3 and incubated with gentle agitation at room temperature in the dark for 30 min. Excess IA solution was removed and plugs incubated for 15 min with 200μl of 50 mM NH_4HCO_3 at room temperature. A volume of 200 μl of 100% acetonitrile was added to this solution and incubated for 15 min at room temperature. Solvent was removed and gel plugs were allowed to dry for 30 min at RT under a flow hood. Plugs were rehydrated with 20 ng/μl of modified trypsin (Promega, Madison, WI, USA) in 50 mM NH_4HCO_3 in a shaking incubator overnight at 37 °C. Enough trypsin solution was added in order to completely submerge the gel plugs.

Nano LC-Mass spectrometry

Samples desalted with C_{18} Zip Tips were reconstituted in 10 μL 5% ACN/0.1% FA and analyzed by a nanoAcquity (Waters, Milford, MA)-LTQ Orbitrap XL (Thermo Scientific,

San Jose, CA) system in data dependent scan mode. An in-house packed capillary column (0.1 × 130 mm column packed with 3.6 μm, 200Å XB-C18) and a gradient with 0.1% FA and ACN/0.1% FA at 200 nL/min were used for separation. The MS spectra were acquired by the orbitrap at 30,000 resolution and MS/MS spectra of the six most intense ions in MS scan were obtained by the orbitrap at 7,500 resolution. Data files from each sample were searched against the most current version of the Swiss-Prot database by SEQUEST (Proteome Discoverer v1.4, Thermo Scientific). At least two high-confidence peptide matches were required for protein identification (false discovery rate <1%). Proteins matched with the same peptides are reported as one protein group. Resultant MS/MS data was initially verified by comparison of the identified protein's expected molecular weight and isoelectric point to that of the extracted plug from the 2-D gel.

Results

Proteomics is a method to analyze the proteome of a cell and it is an excellent tool for the screening of the most abundant protein differences in physiological as well as pathological conditions [26, 27]. The current study measured proteomic differences in the frontal cortex from DS cases with and without AD compared to non-DS cases (young and older) to characterize the changes in protein expression induced by age, DS and AD neuropathology. Furthermore, we investigated how these three variables may act in concert on changes of protein profile.

The study contains a four-way comparison, comprising four distinct groups of protein samples to be analyzed: 1) young healthy control group vs young DS group, 2) old healthy control group vs DS AD group, 3) DS group vs DSAD group, and 4) young healthy control group (≥ 40 years) vs old healthy control group (> 40 years). Each sample was processed by 2D electrophoresis and all the gel maps obtained were compared at the same time with a dedicated software program (PD-Quest, BioRad). Then, all the samples were divided into four groups according to age or the presence of DS and/or AD. All the statistically significant spots were excised from the gels, digested with trypsin, and the resulting peptides sequenced by MS/MS analyses. All protein identifications were consistent with comparison of protein positions on the gel with MW and pI from databases.

Young DS *vs* Young control

The comparison between the young control group and the young DS group was used to detect changes in protein levels associated with DS. We found seven proteins with a differential expression pattern in frontal cortex from young control *vs.* young DS: Ras-related protein Rab-3A (Rab-3A); guanine nucleotide-binding protein g (I)/G (S) subunit beta-1 (GNB1); apolipoprotein E (APO E); transitional endoplasmic reticulum ATPase (TER ATPase); pyridoxal phosphate phosphatase (PLP phosphatase); and α-enolase showed significantly decreased expression in young DS compared to young control group. The only protein with elevated levels in the young DS group compared with the young control group was malate dehydrogenase mitochondrial (MDH2) (Table 2 A, Fig.1 A).

DSAD *vs* Old control

When the group of old control was compared to DS with AD neuropathology group, three proteins were identified that were decreased in the latter group: rho GDP-dissociation inhibitor 1 (Rho GDI1); dihydropyrimidinase-related protein 2 (DRP-2), also called collapsin response mediator protein (CRMP-2); astrocytic phosphoprotein PEA-15 (PEA15) (Table 2 B, Fig. 1 B).

Young DS *vs* DSAD

We compared the frontal cortex proteome from the young DS group and the DS group with AD to detect changes in the proteome associated with AD neuropathology. Three proteins were identified with increased levels in young DS compared to the DSAD group: elongation factor Tu mitochondrial (EF-Tu); thioredoxin-dependent peroxide reductase mitochondrial (PRDX3); and α-enolase (Table 3 A, Fig. 1 C).

Young CTR vs old control

The comparison between young control group and old control group allowed the identification of proteins with altered expression associated with aging. Ten proteins were identified with differential levels: tubulin-folding cofactor B; beta-soluble NSF attachment protein; fructose-bisphosphatealdolase C; actin-related protein 2/3 complex subunit 2; transitional endoplasmic reticulum ATPase; and pyridoxal phosphate phosphatase were identified with increased levels in young control compared to old control. In contrast, dynamin-1, dihydropyrimidinase-related protein 2 (DRP2), glutamate dehydrogenase 1 (GDH-1), and ATP-synthase subunit alpha showed decreased expression in young control group compared to the old control group (Table 3 B, Fig. 1 D).

Discussion

Down syndrome (DS) is one of the most common causes of intellectual disability and is caused by trisomy of chromosome 21 [8]. This abnormal chromosomal condition leads to a wide heterogeneity in DS phenotypes [28], among which age-associated neuropathology is a consistent feature [20]. A high risk for developing Alzheimer disease (AD) dementia in people who have DS over the age of 50 years has been demonstrated [7, 12]. Several factors may contribute to AD dementia in DS, including the over-expression of amyloid precursor protein (APP) on chromosome 21, responsible for the early deposition of Aβ [12]. Further, oxidative damage, mainly produced by SOD-1 over-expression, and by the enhanced deposition of Aβ, may in combination exacerbate the development of AD in DS dysfunction [17, 18].

To shed light on cellular pathways compromised in DS, both before and after the presence of AD neuropathology, we analyzed the frontal cortex proteome in DS with and without AD neuropathology compared to age-matched healthy cases applying a proteomics approach. Comparing frontal cortex from subjects with DS and DS with AD neuropathology with respective age-matched control groups, we investigated the impact that age, DS and AD neuropathology may have on protein expression levels (Fig. 2).

The comparison of the frontal cortex proteome from the young DS group with that of DS with AD neuropathology allowed the identification of how the proteome changes when AD neuropathology appears in DS brains. We found three proteins: EF-Tu, PRDX3, and α-enolase, which are involved in protein synthesis, antioxidant function, and energy metabolism, respectively with decreased expression in DS group with AD neuropathology.

Elongation factor Tu (EF-Tu) is involved in the protein synthesis machinery. Human mitochondrial EF-Tu is a nuclear-encoded protein involved in the synthesis of proteins, which are part of the electron transport chain and ATP synthetase [29]. The current study reports a significant decrease of EF-Tu levels in DS frontal cortex AD neuropathology is present. EF-Tu was also found to be significantly down-regulated in the cortex from a fetus with DS in the second trimester of gestation compared to healthy controls [30]. Also one of the first models for DS, transgenic mice expressing wild-type hSOD1 (Tg-SOD1) showed significantly decreased hippocampal of EF-Tu levels compared to wild type mice [31]. Taken together these data suggest that impairment of the protein synthesis complex, mainly those essential for energy and metabolism, begins at early stages and gets worse with time in particular with the appearance of AD neuropathology. Furthermore, in an early stage of AD, mild cognitive impairment (MCI), EF-Tu was found excessively bound to the lipid peroxidation product 4-hydroxy-2-nonenal (HNE) compared to control leading to protein dysfunction and neuronal death [32]. The reduced expression of EF-tu in DS with AD neuropathology compared to young DS may contribute to the development of the clinical signs of dementia and neuropathological features of AD.

In accordance with the extensive literature supporting the involvement of oxidative stress in the pathogenesis of several neurodegenerative diseases, such as DS and AD [16, 17, 33], our study showed an antioxidant protein, thioredoxin-dependent peroxide reductase (PRDX3), significantly decreased in frontal cortex of DS with AD-neuropathology compared to DS without AD neuropathology. PRDX3 is primarily localized to the matrix of mitochondria and regulates physiological levels of H_2O_2, protecting cells from the apoptosis-inducing effects of high levels of H_2O_2 [34]. Previous work from Lubec's group agree with our data, reporting PRDX3 levels significantly are reduced in the cortex of both DS and AD patients [35]. Mitochondrial impairment has been reported in DS and AD [36, 37] and could cause low levels of PRDX3 and contribute to the differences in levels of this important antioxidant enzyme observed in our study between young DS and DS with AD neuropathology. In turn, low levels of PRDX3, may be related to altered capacity to counteract oxidative damage, and consequently potentially responsible for onset of neurodegeneration in DS.

Dysfunction of energy metabolism is well known to be closely related to several age-related neurodegenerative disorders. α-enolase, a glycolytic enzyme, has been found to be significantly oxidatively modified in individuals with mild cognitive impairment (MCI) [38, 39], in AD, and in various models of neurodegenerative diseases suggesting its central role in these disorders [40, 41]. Intriguingly, in our study α-enolase levels in brain are decreased when comparing the young control group to young DS, and to DS with AD neuropathology. Furthermore, our data were in line with a recent redox proteomics study that has revealed that α-enolase was significantly oxidized in the DS group with and without AD neuropathology compared to relative aged-matched samples [23]. Several impaired

metabolic functions in DS have been reported including the deterioration of glucose metabolism [42, 43]. Furthermore, Lubec's group has demonstrated, by proteomics techniques, alteration in protein expression of some enzymes involved in intermediary metabolism in fetal DS brain suggesting that metabolism is compromised during prenatal development in DS individuals [44]. In addition, Schapiro et al. [45], found that elderly demented DS subjects showed similar rates of glucose metabolism, evaluated by positron emission tomography (PET) to those described previously in AD patients. Our results, supported by the literature, further suggest the involvement of impaired energy metabolism in aging and the development of AD in DS. Enolase is more than a glycolytic enzyme: enolase can lead to activation of plasminogen, which produces plasmin that can degrade Aβ, and to pro-survival pathways involving ERK1/2 [38]. Thus, oxidative dysfunction of enolase can have profound detrimental effects in DS brain.

When comparing the frontal cortex proteome in the young DS group with that from age-matched healthy cases, we gained insights into pathways compromised in younger individuals with DS and that potentially may be promising targets for therapeutic intervention. In addition to α-enolase, discussed above, an additional protein involved in energy metabolism, malate dehydrogenase (MDH), was over-expressed in young DS compared to controls. Encoded by MDH2 gene, MDH is the final enzyme in the mitochondrial tricarboxylic acid (TCA) cycle. MDH activity is elevated in the brains of subjects with AD [46, 47]. This enzyme was also found to be more nitrated in AD than in control subjects in the hippocampus of MCI subjects [48], but less nitrated in caloric-restricted rat hippocampus from aged rodents [37]. Further, MDH was also found to be covalently bound by HNE in early AD in the inferior parietal lobule compared to similarly aged control brain [49]. Since increased activity of MDH occurs, it was proposed that HNE modifications of brain MDH in early AD leads to conformational changes of the MDH active site. Recently, we analyzed the oxidative and nitrosative stress markers in the same samples considered in this study. Among all the oxidative and nitrosative stress markers, only HNE-bound proteins level was significantly increased in young DS compared to the other analyzed groups [24]. Furthermore, also cytosolic MDH analyzed by redox proteomics in the same samples used in this study has showed higher levels of oxidation in DS group with AD neuropathology compared to old healthy control group [23]. Therefore, we could speculate that increased MDH in frontal cortex of young DS could be related to HNE-induced modifications of the active site of this protein. Our findings related to both MDH and α-enolase are consistent with involvement of mitochondrial dysfunction and oxidative damage in DS.

Lower levels of apolipoprotein E (ApoE) in frontal cortex in young DS cases compared to the age-matched control group were observed. ApoE is an apolipoprotein that in the brain plays an important role in the regulation of cholesterol and Aβ transport and clearance. ApoE plays a role in neuronal plasticity, neurite outgrowth, and synaptogenesis [50]. Studies suggest that ApoE-null mice develop mild to severe spatial learning and memory deficits [51, 52] and elevated oxidative stress [53]. Furthermore, it was demonstrated that ApoE deficient mice were also more susceptible to neurodegeneration than their wild-type counterparts. Indeed, the deficiency of ApoE significantly exacerbates the formation of Aβ-like deposition as an early event before any CNS defects [54]. The decreased level of ApoE

in young DS could contribute to the earlier age of onset of Aβ accumulation in DS and have detrimental effects on neuron function [55].

Another protein that was found significantly decreased in the frontal cortex from young DS people compared to the age-matched healthy subjects is Ras-related protein (Rab-3A). This protein is specifically expressed in the brain, localized at presynaptic level and it regulates the synaptic vesicle exocytosis of neurotrasmettitors [56]. In a previous work, Reddy et al. have shown a substantial loss of both presynaptic vesicle proteins and postsynaptic proteins, including Rab 3A, in brains from AD patients compared to age-matched control subjects. Furthermore, they found that the presynaptic proteins synaptophysin and Rab 3A and the postsynaptic protein synaptopodin were the most down regulated in AD compared to healthy subjects [57]. All together, these data suggest that synapse loss could be an early event in DS brain and may be related to cognitive impairment, neurodegeneration and onset of the AD condition in Down syndrome.

In addition to the above, an intriguing result of our study is suggested by the protein, transitional endoplasmic reticulum ATPase (TER ATPase), also called valosin-containing protein (VCP), essential for autophagic processes [58]. Autophagy is a major degradative pathway for organelles and proteins [59]. Neurons are particularly dependent on autophagy for their survival [60, 61]. Changes in autophagy has been implicated in the pathogenesis of several neurodegenerative diseases, such as PD, AD and DS [62–64]. In particular, enhanced Aβ production leads to lysosomal dysfunction, which is responsible independently for neuronal dysfunction, further Aβ accumulation, and consequently cell death [65, 66]. In our study, VCP levels were over-expressed in frontal cortex of young controls compared to frontal cortex from both old controls and DS subjects, consistent with previous data about early autophagy impairment in young DS. Therefore, means for restoration of autophagy may be a promising therapeutic strategy to slow or reduce the development of AD in DS.

In this study we gained insights into pathological mechanisms that may be involved in the DS phenotype before the onset of AD neuropathology and after AD pathology is present. These altered pathways could be useful as potential targets for therapeutics. As shown in Figure 2, α-enolase plays a critical role in DS and in DSAD and may be another promising therapeutic target for individuals with DS. Further studies to investigate this possibility and to identify brain proteins that are specifically oxidatively modified in young DS and DS with AD neuropathology may provide insights into oxidative stress in DS and DSAD and are ongoing in our laboratories [23].

Acknowledgments

This work was supported in part by grants from NIH to D.A.B [AG-05119], and to E.H. from NICHD [HD-064993]. Additional funding was provided by NIH to the UCI ADRC (P50 AG16573) and to the UK ADC (P30 AG028383). Human tissue obtained from NICHD Brain and Tissue Bank for Developmental Disorders at the University of Maryland, Baltimore, MD, was under contract HHSN275200900011C, Ref. No. N01-HD-9-0011.

References

1. Lejeune J. Pathogenesis of mental deficiency in trisomy 21. Am J Med Genet Suppl. 1990; 7:20–30. [PubMed: 2149947]

2. Epstein CJ. Developmental genetics. Experientia. 1986; 42:1117–1128. [PubMed: 3021509]
3. Mann DM, Esiri MM. The pattern of acquisition of plaques and tangles in the brains of patients under 50 years of age with Down's syndrome. J Neurol Sci. 1989; 89:169–179. [PubMed: 2522541]
4. Wisniewski K, Howe J, Williams DG, Wisniewski HM. Precocious aging and dementia in patients with Down's syndrome. Biol Psychiatry. 1978; 13:619–627. [PubMed: 153156]
5. Lai F, Williams RS. A prospective study of Alzheimer disease in Down syndrome. Archives of neurology. 1989; 46:849–853. [PubMed: 2527024]
6. Tyrrell J, Cosgrave M, McCarron M, McPherson J, Calvert J, Kelly A, McLaughlin M, Gill M, Lawlor BA. Dementia in people with Down's syndrome. International journal of geriatric psychiatry. 2001; 16:1168–1174. [PubMed: 11748777]
7. Bush A, Beail N. Risk factors for dementia in people with down syndrome: issues in assessment and diagnosis. American journal of mental retardation : AJMR. 2004; 109:83–97. [PubMed: 15000668]
8. Patterson D. Molecular genetic analysis of Down syndrome. Hum Genet. 2009; 126:195–214. [PubMed: 19526251]
9. Das I, Reeves RH. The use of mouse models to understand and improve cognitive deficits in Down syndrome. Disease models & mechanisms. 2011; 4:596–606. [PubMed: 21816951]
10. Lahiri DK, Ge YW, Maloney B, Wavrant-De Vrieze F, Hardy J. Characterization of two APP gene promoter polymorphisms that appear to influence risk of late-onset Alzheimer's disease. Neurobiology of aging. 2005; 26:1329–1341. [PubMed: 16243604]
11. Suh YH, Checler F. Amyloid precursor protein, presenilins, and alpha-synuclein: molecular pathogenesis and pharmacological applications in Alzheimer's disease. Pharmacological reviews. 2002; 54:469–525. [PubMed: 12223532]
12. Lott IT, Head E. Down syndrome and Alzheimer's disease: a link between development and aging. Mental retardation and developmental disabilities research reviews. 2001; 7:172–178. [PubMed: 11553933]
13. Lemere CA, Blusztajn JK, Yamaguchi H, Wisniewski T, Saido TC, Selkoe DJ. Sequence of deposition of heterogeneous amyloid beta-peptides and APO E in Down syndrome: implications for initial events in amyloid plaque formation. Neurobiol Dis. 1996; 3:16–32. [PubMed: 9173910]
14. Leverenz JB, Raskind MA. Early amyloid deposition in the medial temporal lobe of young Down syndrome patients: a regional quantitative analysis. Experimental neurology. 1998; 150:296–304. [PubMed: 9527899]
15. Jovanovic SV, Clements D, MacLeod K. Biomarkers of oxidative stress are significantly elevated in Down syndrome. Free radical biology & medicine. 1998; 25:1044–1048. [PubMed: 9870557]
16. Perluigi M, Butterfield DA. Oxidative Stress and Down Syndrome: A Route toward Alzheimer-Like Dementia. Current gerontology and geriatrics research. 2012; 2012:724–904.
17. Zana M, Janka Z, Kalman J. Oxidative stress: a bridge between Down's syndrome and Alzheimer's disease. Neurobiology of aging. 2007; 28:648–676. [PubMed: 16624449]
18. Lott IT, Head E, Doran E, Busciglio J. Beta-amyloid, oxidative stress and down syndrome. Current Alzheimer research. 2006; 3:521–528. [PubMed: 17168651]
19. Head E, Silverman W, Patterson D, Lott IT. Aging and down syndrome. Current gerontology and geriatrics research. 2012; 2012:412–536.
20. Schupf N, Sergievsky GH. Genetic and host factors for dementia in Down's syndrome. Br J Psychiatry. 2002; 180:405–410. [PubMed: 11983636]
21. Sun Y, Dierssen M, Toran N, Pollak DD, Chen WQ, Lubec G. A gel-based proteomic method reveals several protein pathway abnormalities in fetal Down syndrome brain. Journal of proteomics. 2011; 74:547–557. [PubMed: 21262400]
22. Cheon MS, Fountoulakis M, Dierssen M, Ferreres JC, Lubec G. Expression profiles of proteins in fetal brain with Down syndrome. Journal of neural transmission. Supplementum. 2001:311–319. [PubMed: 11771754]
23. Di Domenico F, Pupo G, Tramutola A, Giorgi A, Schinina ME, Coccia R, Head E, Butterfield DA, Perluigi M. Redox proteomics analysis of HNE-modified proteins in Down syndrome brain: clues for understanding the development of Alzheimer disease. Free radical biology & medicine. 2014; 71C:270–280. [PubMed: 24675226]

24. Cenini G, Dowling AL, Beckett TL, Barone E, Mancuso C, Murphy MP, Levine H 3rd, Lott IT, Schmitt FA, Butterfield DA, Head E. Association between frontal cortex oxidative damage and beta-amyloid as a function of age in Down syndrome. Biochim Biophys Acta. 2012; 1822:130–138. [PubMed: 22009041]
25. Di Domenico F, Sultana R, Barone E, Perluigi M, Cini C, Mancuso C, Cai J, Pierce WM, Butterfield DA. Quantitative proteomics analysis of phosphorylated proteins in the hippocampus of Alzheimer's disease subjects. Journal of proteomics. 2011; 74:1091–1103. [PubMed: 21515431]
26. Butterfield DA, Sultana R. Redox proteomics identification of oxidatively modified brain proteins in Alzheimer's disease and mild cognitive impairment: insights into the progression of this dementing disorder. Journal of Alzheimer's disease : JAD. 2007; 12:61–72.
27. Shi M, Caudle WM, Zhang J. Biomarker discovery in neurodegenerative diseases: a proteomic approach. Neurobiology of Disease. 2009; 35:157–164. [PubMed: 18938247]
28. Deutsch S, Lyle R, Dermitzakis ET, Attar H, Subrahmanyan L, Gehrig C, Parand L, Gagnebin M, Rougemont J, Jongeneel CV, Antonarakis SE. Gene expression variation and expression quantitative trait mapping of human chromosome 21 genes. Human molecular genetics. 2005; 14:3741–3749. [PubMed: 16251198]
29. Ling M, Merante F, Chen HS, Duff C, Duncan AM, Robinson BH. The human mitochondrial elongation factor tu (EF-Tu) gene: cDNA sequence, genomic localization, genomic structure, and identification of a pseudogene. Gene. 1997; 197:325–336. [PubMed: 9332382]
30. Freidl M, Gulesserian T, Lubec G, Fountoulakis M, Lubec B. Deterioration of the transcriptional, splicing and elongation machinery in brain of fetal Down syndrome. Journal of neural transmission. Supplementum. 2001:47–57. [PubMed: 11771760]
31. Shin JH, London J, Le Pecheur M, Hoger H, Pollak D, Lubec G. Aberrant neuronal and mitochondrial proteins in hippocampus of transgenic mice overexpressing human Cu/Zn superoxide dismutase 1. Free radical biology & medicine. 2004; 37:643–653. [PubMed: 15288122]
32. Reed T, Perluigi M, Sultana R, Pierce WM, Klein JB, Turner DM, Coccia R, Markesbery WR, Butterfield DA. Redox proteomic identification of 4-hydroxy-2-nonenal-modified brain proteins in amnestic mild cognitive impairment: insight into the role of lipid peroxidation in the progression and pathogenesis of Alzheimer's disease. Neurobiol Dis. 2008; 30:107–120. [PubMed: 18325775]
33. Butterfield DA, Drake J, Pocernich C, Castegna A. Evidence of oxidative damage in Alzheimer's disease brain: central role for amyloid beta-peptide. Trends in molecular medicine. 2001; 7:548–554. [PubMed: 11733217]
34. Rhee SG, Kang SW, Netto LE, Seo MS, Stadtman ER. A family of novel peroxidases, peroxiredoxins. BioFactors. 1999; 10:207–209. [PubMed: 10609884]
35. Kim SH, Fountoulakis M, Cairns N, Lubec G. Protein levels of human peroxiredoxin subtypes in brains of patients with Alzheimer's disease and Down syndrome. J Neural Transm Suppl. 2001:223–235. [PubMed: 11771746]
36. Pallardo FV, Degan P, d'Ischia M, Kelly FJ, Zatterale A, Calzone R, Castello G, Fernandez-Delgado R, Dunster C, Lloret A, Manini P, Pisanti MA, Vuttariello E, Pagano G. Multiple evidence for an early age pro-oxidant state in Down Syndrome patients. Biogerontology. 2006; 7:211–220. [PubMed: 16612664]
37. Poon HF, Shepherd HM, Reed TT, Calabrese V, Stella AM, Pennisi G, Cai J, Pierce WM, Klein JB, Butterfield DA. Proteomics analysis provides insight into caloric restriction mediated oxidation and expression of brain proteins associated with age-related impaired cellular processes: Mitochondrial dysfunction, glutamate dysregulation and impaired protein synthesis. Neurobiology of aging. 2006; 27:1020–1034. [PubMed: 15996793]
38. Butterfield DA, Lange ML. Multifunctional roles of enolase in Alzheimer's disease brain: beyond altered glucose metabolism. Journal of neurochemistry. 2009; 111:915–933. [PubMed: 19780894]
39. Butterfield DA, Poon HF, St Clair D, Keller JN, Pierce WM, Klein JB, Markesbery WR. Redox proteomics identification of oxidatively modified hippocampal proteins in mild cognitive impairment: insights into the development of Alzheimer's disease. Neurobiol Dis. 2006; 22:223–232. [PubMed: 16466929]

40. Castegna A, Aksenov M, Thongboonkerd V, Klein JB, Pierce WM, Booze R, Markesbery WR, Butterfield DA. Proteomic identification of oxidatively modified proteins in Alzheimer's disease brain. Part II: dihydropyrimidinase-related protein 2, alpha-enolase and heat shock cognate 71. Journal of neurochemistry. 2002; 82:1524–1532. [PubMed: 12354300]

41. Perluigi M, Poon HF, Maragos W, Pierce WM, Klein JB, Calabrese V, Cini C, De Marco C, Butterfield DA. Proteomic analysis of protein expression and oxidative modification in r6/2 transgenic mice: a model of Huntington disease. Molecular & cellular proteomics : MCP. 2005; 4:1849–1861. [PubMed: 15968004]

42. Schapiro MB, Haxby JV, Grady CL, Duara R, Schlageter NL, White B, Moore A, Sundaram M, Larson SM, Rapoport SI. Decline in cerebral glucose utilisation and cognitive function with aging in Down's syndrome. Journal of neurology, neurosurgery, and psychiatry. 1987; 50:766–774.

43. Dani A, Pietrini P, Furey ML, McIntosh AR, Grady CL, Horwitz B, Freo U, Alexander GE, Schapiro MB. Brain cognition and metabolism in Down syndrome adults in association with development of dementia. Neuroreport. 1996; 7:2933–2936. [PubMed: 9116213]

44. Bajo M, Fruehauf J, Kim SH, Fountoulakis M, Lubec G. Proteomic evaluation of intermediary metabolism enzyme proteins in fetal Down's syndrome cerebral cortex. Proteomics. 2002; 2:1539–1546. [PubMed: 12442254]

45. Schapiro MB, Haxby JV, Grady CL. Nature of mental retardation and dementia in Down syndrome: study with PET, CT, and neuropsychology. Neurobiology of aging. 1992; 13:723–734. [PubMed: 1491738]

46. Bubber P, Haroutunian V, Fisch G, Blass JP, Gibson GE. Mitochondrial abnormalities in Alzheimer brain: mechanistic implications. Ann Neurol. 2005; 57:695–703. [PubMed: 15852400]

47. Korolainen MA, Goldsteins G, Nyman TA, Alafuzoff I, Koistinaho J, Pirttila T. Oxidative modification of proteins in the frontal cortex of Alzheimer's disease brain. Neurobiology of aging. 2006; 27:42–53. [PubMed: 16298240]

48. Sultana R, Reed T, Perluigi M, Coccia R, Pierce WM, Butterfield DA. Proteomic identification of nitrated brain proteins in amnestic mild cognitive impairment: a regional study. Journal of cellular and molecular medicine. 2007; 11:839–851. [PubMed: 17760844]

49. Reed TT, Pierce WM, Markesbery WR, Butterfield DA. Proteomic identification of HNE-bound proteins in early Alzheimer disease: Insights into the role of lipid peroxidation in the progression of AD. Brain research. 2009; 1274:66–76. [PubMed: 19374891]

50. Hauser PS, Narayanaswami V, Ryan RO. Apolipoprotein E: from lipid transport to neurobiology. Progress in lipid research. 2011; 50:62–74. [PubMed: 20854843]

51. Champagne D, Dupuy JB, Rochford J, Poirier J. Apolipoprotein E knockout mice display procedural deficits in the Morris water maze: analysis of learning strategies in three versions of the task. Neuroscience. 2002; 114:641–654. [PubMed: 12220566]

52. Oitzl MS, Mulder M, Lucassen PJ, Havekes LM, Grootendorst J, de Kloet ER. Severe learning deficits in apolipoprotein E-knockout mice in a water maze task. Brain research. 1997; 752:189–196. [PubMed: 9106456]

53. Lauderback CM, Hackett JM, Keller JN, Varadarajan S, Szweda L, Kindy M, Markesbery WR, Butterfield DA. Vulnerability of synaptosomes from apoE knock-out mice to structural and oxidative modifications induced by A beta(1–40): implications for Alzheimer's disease. Biochemistry. 2001; 40:2548–2554. [PubMed: 11327877]

54. Robertson TA, Dutton NS, Martins RN, Roses AD, Kakulas BA, Papadimitriou JM. Beta-amyloid protein-containing inclusions in skeletal muscle of apolipoprotein-E-deficient mice. The American journal of pathology. 1997; 150:417–427. [PubMed: 9033257]

55. Head E, Lott IT. Down syndrome and beta-amyloid deposition. Current opinion in neurology. 2004; 17:95–100. [PubMed: 15021233]

56. Weber E, Jilling T, Kirk KL. Distinct functional properties of Rab3A and Rab3B in PC12 neuroendocrine cells. The Journal of biological chemistry. 1996; 271:6963–6971. [PubMed: 8636125]

57. Reddy PH, Mani G, Park BS, Jacques J, Murdoch G, Whetsell W Jr, Kaye J, Manczak M. Differential loss of synaptic proteins in Alzheimer's disease: implications for synaptic dysfunction. Journal of Alzheimer's disease : JAD. 2005; 7:103–117. discussion 173–180;

58. Ju JS, Fuentealba RA, Miller SE, Jackson E, Piwnica-Worms D, Baloh RH, Weihl CC. Valosin-containing protein (VCP) is required for autophagy and is disrupted in VCP disease. The Journal of cell biology. 2009; 187:875–888. [PubMed: 20008565]

59. Klionsky DJ. The molecular machinery of autophagy and its role in physiology and disease. Seminars in cell & developmental biology. 2010; 21:663. [PubMed: 20430106]

60. Hara T, Nakamura K, Matsui M, Yamamoto A, Nakahara Y, Suzuki-Migishima R, Yokoyama M, Mishima K, Saito I, Okano H, Mizushima N. Suppression of basal autophagy in neural cells causes neurodegenerative disease in mice. Nature. 2006; 441:885–889. [PubMed: 16625204]

61. Komatsu M, Waguri S, Chiba T, Murata S, Iwata J, Tanida I, Ueno T, Koike M, Uchiyama Y, Kominami E, Tanaka K. Loss of autophagy in the central nervous system causes neurodegeneration in mice. Nature. 2006; 441:880–884. [PubMed: 16625205]

62. Nixon RA. Endosome function and dysfunction in Alzheimer's disease and other neurodegenerative diseases. Neurobiology of aging. 2005; 26:373–382. [PubMed: 15639316]

63. Nixon RA, Cataldo AM. Lysosomal system pathways: genes to neurodegeneration in Alzheimer's disease. Journal of Alzheimer's disease : JAD. 2006; 9:277–289.

64. Di Domenico F, Sultana R, Ferree A, Smith K, Barone E, Perluigi M, Coccia R, Pierce W, Cai J, Mancuso C, Squillace R, Wiengele M, Dalle-Donne I, Wolozin B, Butterfield DA. Redox proteomics analyses of the influence of co-expression of wild-type or mutated LRRK2 and Tau on C. elegans protein expression and oxidative modification: relevance to Parkinson disease. Antioxidants & redox signaling. 2012; 17:1490–1506. [PubMed: 22315971]

65. Jiang Y, Mullaney KA, Peterhoff CM, Che S, Schmidt SD, Boyer-Boiteau A, Ginsberg SD, Cataldo AM, Mathews PM, Nixon RA. Alzheimer's-related endosome dysfunction in Down syndrome is Abeta-independent but requires APP and is reversed by BACE-1 inhibition. Proceedings of the National Academy of Sciences of the United States of America. 2010; 107:1630–1635. [PubMed: 20080541]

66. Nixon RA, Yang DS. Autophagy failure in Alzheimer's disease--locating the primary defect. Neurobiol Dis. 2011; 43:38–45. [PubMed: 21296668]

Highlights

- Down syndrome patients develop Alzheimer disease-like pathology after 40 years of age
- DS patients become demented after development of AD-like pathology
- Proteomics revealed altered levels of key brain proteins in DS with AD compared to DS
- New insights into mechanisms engaged prior and after AD pathology in DS have emerged

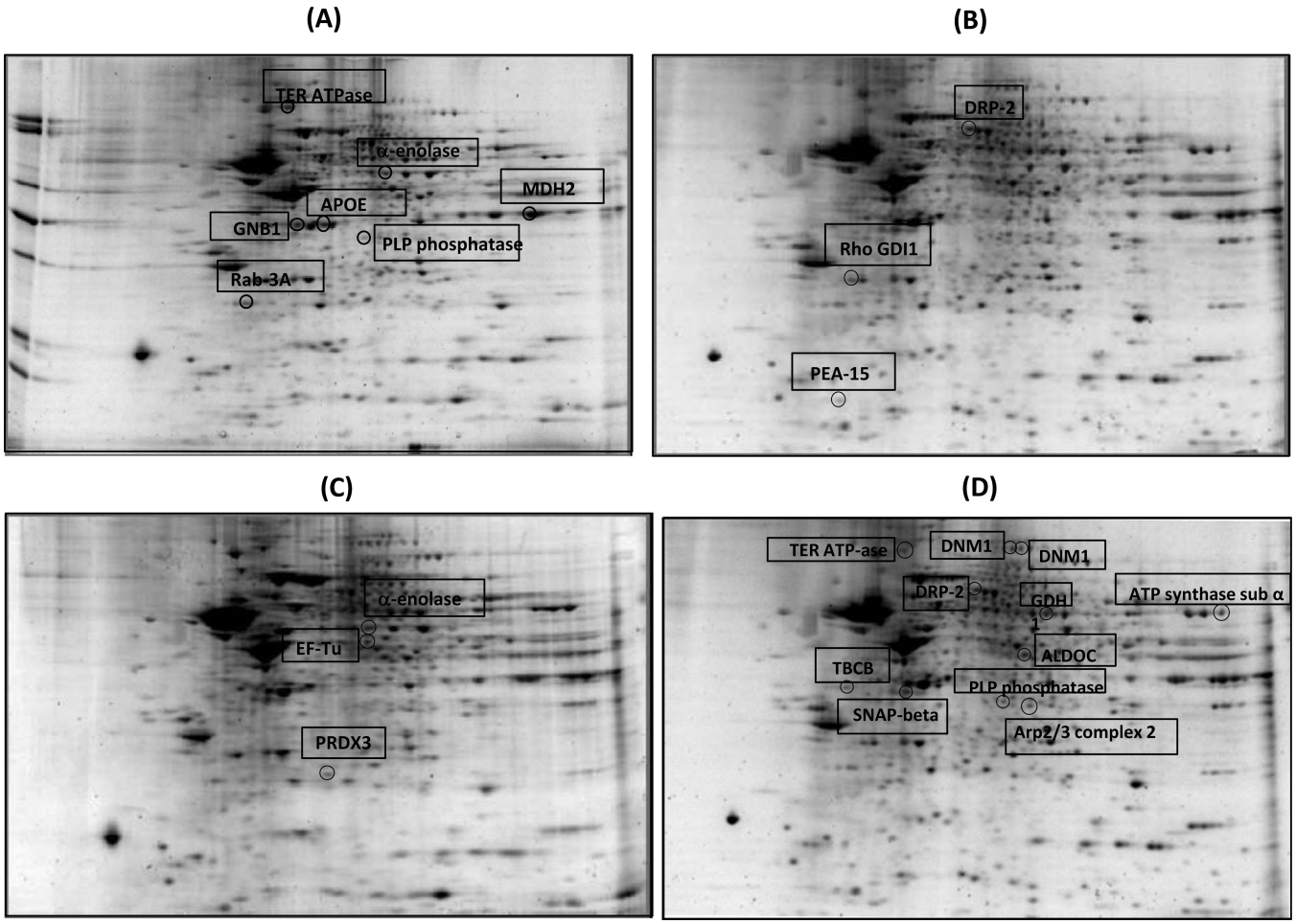

Fig. 1. 2D protein expression maps

Proteomic profile of representative 2D-gels with proteins differently expressed in four groups of matching: young healthy CTR group vs young DS group (A), old healthy CTR group vs DS subjects with AD-like dementia (B), DS group vs DSAD group (C), and young healthy CTR group vs old healthy CTR group (D). The identified proteins by mass spectrometry are reported.

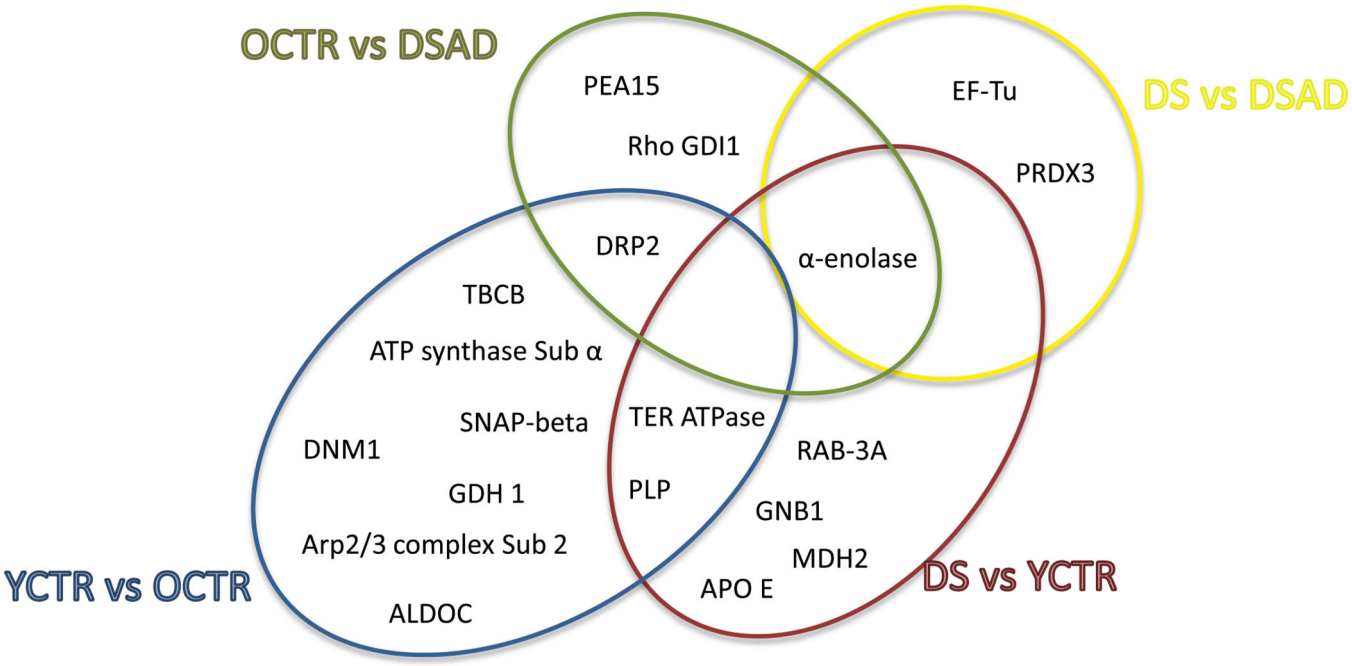

Fig. 2. Venn diagram on overlapping proteins between the four different groups of analysis

Table 1

Case Demographics

Group	n	Gender (M/F)	Age (SD)	PMI (SD)
YC	6	3/3	13.10 (15.30)	17.50 (6.50)
OC	6	4/2	53.00 (8.50)	10.80 (5.90)
DS	6	5/1	11.01 (10.90)	17.00 (4.50)
DS/AD	6	3/3	45.60 (3.90)	9.39 (6.80)

PMI, postmortem interval; **SD**, standard deviation; **M**, male; **F**, female; **DS**, Down Syndrome; **AD**, Alzheimer disease; **DS/AD**, Down syndrome with Alzheimer disease neuropathology; **YC**, Young Control; **OC**, Old Control

Table 2

Summary of the proteins with different levels identified by proteomics in DS vs young Control frontal cortex (A); and in DSAD vs old Control frontal cortex (B)

Protein	SwissProt Accession	Fold-change	*p*-value	Function
(A) DS vs. young Control				
Rab-3A	P20336	3.50↓	0.003	Synaptic vesicle trafficking
GNB1	P62873	1.41↓	0.048	Signaling transduction
APO E	P02649	1.75↓	0.033	Lipoprotein metabolism
TER ATPase	P55072	5.67↓	0.014	Vesicular trafficking
PLP phosphatase	Q96GD0	3.74↓	0.036	Coenzyme Vitamin B6
MDH2	P40926	2.30↑	0.026	Energy metabolism
α-enolase	P06733	2.50↓	0.018	Energy metabolism
(B) DSAD vs. old Control				
Rho GDI1	P52665	2.58↓	0.038	Signaling/Regulation
DRP-2	Q16555	3.87↓	0.012	Neuron Structure
PEA15	Q15121	2.22↓	0.040	Astrocytes proliferation

Table 3

Summary of the proteins with different levels identified by proteomics in DS vs DSAD frontal cortex (A); and in young Control vs. old Control frontal cortex (B).

Protein	SwissProt Accession	Fold-change	*p*-value	Function
(A) DS vs. DSAD				
EF-Tu	P49411	2.40↑	0.044	Protein synthesis
PRDX3	P30048	1.50↑	0.048	Mitochondrial antioxidant
α-enolase	P06733	2.45↑	0.010	Energy metabolism
(B) young Control vs. old Control				
TBCB	Q99426	2.50↑	0.023	Structural/Microtubule
SNAP-beta	Q9H115	2.11↑	0.019	Vesicular transport ER-Golgi
TER ATPase	P55072	3.70↑	0.037	Vesicular trafficking
DRP-2	Q16555	3.40↓	0.006	Neuron Structure
GDH 1	P00367	1.60↓	0.008	Energy metabolism
DNM1	Q05193	2.30↓	0.007	Synaptic vesicle endocytosis
ATP synthase Sub α	P25705	1.80↓	0.037	Energy metabolism
ALDOC	P09972	6.40↑	0.029	Energy metabolism
Arp2/3 complex Sub 2	O15144	3.20↑	0.011	Structural/Cytoskeleton
PLP phosphatase	Q96GD0	2.70↑	0.044	Coenzyme Vit B