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Original Contribution

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

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ABSTRACT

Down syndrome (DS) is one of the most common causes of intellectual disability, owing 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.

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 lead 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 [3–5], and clinical signs of dementia become more frequent after 50 years of age [6,7]. Several studies support the hypothesis that the overexpression of genes present on chromosome 21 is responsible for the features of DS, including the development of AD [8,9]. The overexpression of amyloid precursor protein (APP), due to its location on chromosome 21, leads to an enhanced production of β-amyloid (Aβ), the principle component of SP. Mutations in the APP gene are related to familial AD [10], and therefore APP overexpression 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 life, as young as 8 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 reactive oxygen species production, superoxide dismutase (SOD1) is the most relevant, because its upregulation without a similar increase in 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) and Aβ(1–42), 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 eventual 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

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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 10 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 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 nondemented older control cases were obtained from the University of California at 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 DS/AD, 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 postmortem interval (PMI) was different across groups, with the DS/AD 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], redox proteomics [23], and oxidative damage [24].

Sample preparation

Samples from the frontal cortex of non-DS controls, DS, and DS/AD were thawed in lysis buffer (pH 7.4) containing 320 mM sucrose, 1% 1.0 M Tris–HCl (pH 8.8), 0.098 mM $MgCl_2$, 0.076 mM EDTA, and 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, USA). The brains were homogenized by 20 passes in a Wheaton tissue homogenizer, and the resulting homogenate was centrifuged at 14,000*g* for 10 min to remove cellular debris. The supernatant was extracted to determine the total protein concentration by the BCA method (Pierce, Rockford, IL, USA).

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% ampholytes (Bio-Rad, Hercules, CA, USA), and bromophenol blue and placed in agitation for 3 h. Solubilized proteins were then sonicated twice for 30 s. Samples were loaded on 110-mm, pH 3–10, immobilized pH gradient strips in a Bio-Rad isoelectric focusing cell system (Bio-Rad). After 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% sodium dodecyl sulfate, 20% glycerol containing first 2% dithiothreitol and then 2.5% iodoacetamide. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis was performed in Criterion Tris–HCl gels 8–16% (Bio-Rad) at 200 V for 1 h.

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)

M, male; F, female; SD, standard deviation; PMI, postmortem interval; YC, young control; OC, old control; DS, Down syndrome; AD, Alzheimer disease; DS/AD, Down syndrome with Alzheimer disease neuropathology.

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 (λ_{ex} 470 nm, λ_{em} 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 two-dimension (2D) gels was performed. After a significant number of spots was obtained, the automated matching of all spots was 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 *t* test at 95% confidence to identify spots with *p* values of <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 dithiothreitol (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 iodoacetamide in 0.1 M NH_4HCO_3 and incubated with gentle agitation at room temperature in the dark for 30 min. Excess

iodoacetamide solution was removed and plugs were incubated for 15 min with 200 µl 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 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 to completely submerge the gel plugs.

Nano-liquid chromatography–mass spectrometry (MS)

Samples desalted with C_{18} Zip Tips were reconstituted in 10 µl of 5% acetonitrile/0.1% formic acid and analyzed by a nanoAcquity (Waters, Milford, MA, USA)–LTQ Orbitrap XL (Thermo Scientific, San Jose, CA, USA) system in data-dependent scan mode. An in-house-packed capillary column (0.1 × 130-mm column packed with 3.6-µm, 200-Å XB-C_{18}) and a gradient with 0.1% formic acid and acetonitrile/0.1% formic acid 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 the MS scan were obtained by the Orbitrap at 7500 resolution. Data files from each sample were searched against the most current version of the SwissProt database by SEQUEST (Proteome Discoverer version 1.4, 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 were initially verified by comparison of the identified protein's expected molecular weight and isoelectric point to those of the extracted plug from the 2D gel.

Results

Proteomics is a method to analyze the proteome of a cell and it is an excellent tool for screening the most abundant protein differences in physiological as well as pathological conditions [26,27]. This 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 in 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 DS/AD 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, Bio-Rad). 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 and digested with trypsin, and the resulting peptides were sequenced by MS/MS analyses. All protein identifications were consistent with comparison of protein positions on the gel with molecular weight and p*I* from databases.

Young DS vs young controls

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-3 A, guanine nucleotide-binding protein G (I)/G (S) subunit β1 (GNB1), apolipoprotein E (APO E), transitional endoplasmic reticulum ATPase (TER ATPase), pyridoxal phosphate phosphatase (PLP phosphatase), and α-enolase showed significantly decreased expression in the young DS compared to the 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, Fig. 1A).

Table 2
Summary of the proteins with different levels identified by proteomics in DS vs young control frontal cortex and in DS/AD vs old control frontal cortex.

Protein	SwissProt accession	Fold change	*p* value	Function
DS vs young control				
Rab-3 A	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
DS/AD 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	Astrocyte proliferation

DS/AD vs old controls

When the group of old controls was compared to the 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; and astrocytic phosphoprotein PEA-15 (PEA15) (Table 2, Fig. 1B).

Young DS vs DS/AD

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 DS/AD group: elongation factor Tu mitochondrial (EF-Tu), thioredoxin-dependent peroxide reductase mitochondrial (PRDX3), and α-enolase (Table 3, Fig. 1C).

Young control (CTR) vs old CTR

The comparison between the young control group and the 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, β-soluble NSF attachment protein, fructose-bisphosphate aldolase 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, DRP-2, glutamate dehydrogenase 1 (GDH1), and ATP-synthase subunit α showed decreased expression in the young control group compared to the old control group (Table 3, Fig. 1D).

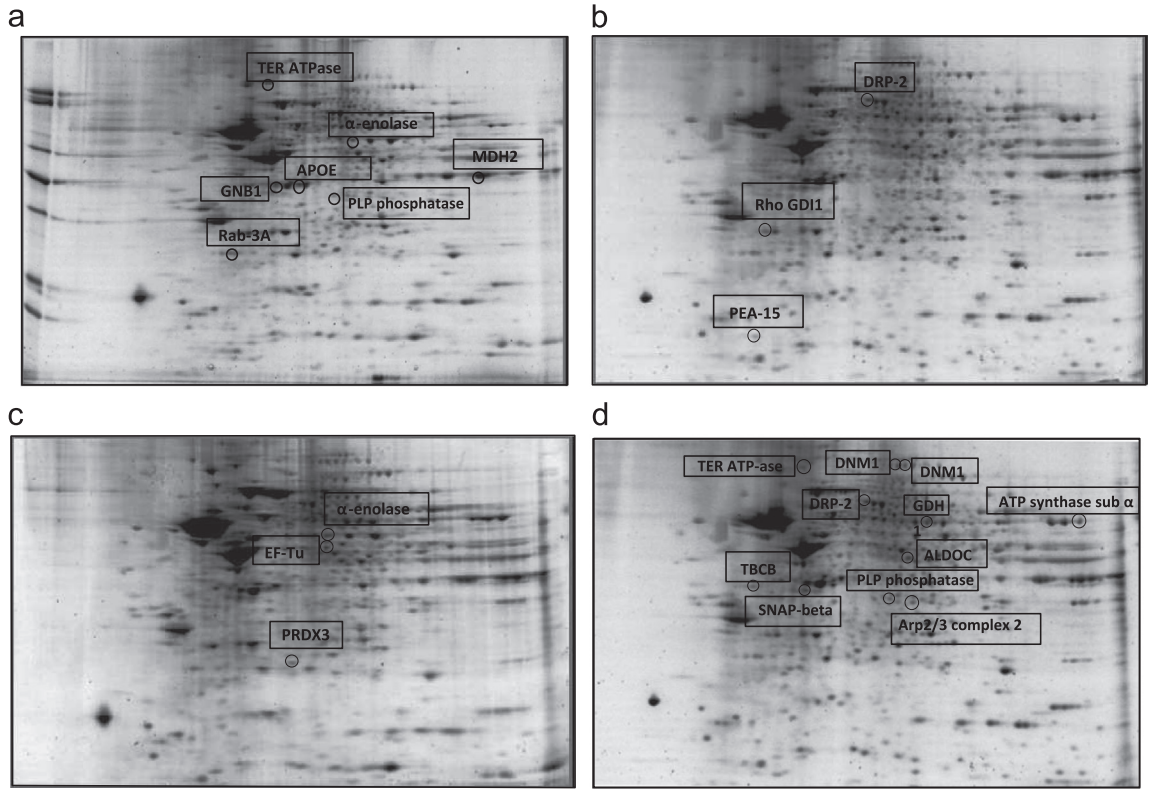

Fig. 1. 2D protein expression maps. Proteomic profiles of representative 2D gels with proteins differentially expressed in four groups comparing (A) young healthy CTR group vs young DS group, (B) old healthy CTR group vs DS subjects with AD-like dementia, (C) DS group vs DS/AD group, and (D) young healthy CTR group vs old healthy CTR group. The proteins identified by mass spectrometry are reported.

Table 3
Summary of the proteins with different levels identified by proteomics in DS vs DS/AD frontal cortex and in young control vs old control frontal cortex.

Protein	SwissProt accession	Fold change	*p* value	Function
DS vs DS/AD				
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
Young control vs old control				
TBCB	Q99426	2.50↑	0.023	Structural/microtubule
SNAP-β	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
GDH1	P00367	1.60↓	0.008	Energy metabolism
DNM1	Q05193	2.30↓	0.007	Synaptic vesicle endocytosis
ATP synthase subunit α	P25705	1.80↓	0.037	Energy metabolism
ALDOC	P09972	6.40↑	0.029	Energy metabolism
Arp2/3 complex subunit 2	O15144	3.20↑	0.011	Structural/cytoskeleton
PLP phosphatase	Q96GD0	2.70↑	0.044	Coenzyme vitamin B

Discussion

Down syndrome 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 AD dementia in people over the age of 50 years who have DS has been demonstrated [7,12]. Several factors may contribute to AD dementia in DS, including the overexpression of APP on chromosome 21, responsible for the early deposition of Aβ [12]. Further, oxidative damage, mainly produced by SOD1 overexpression 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 that of 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,

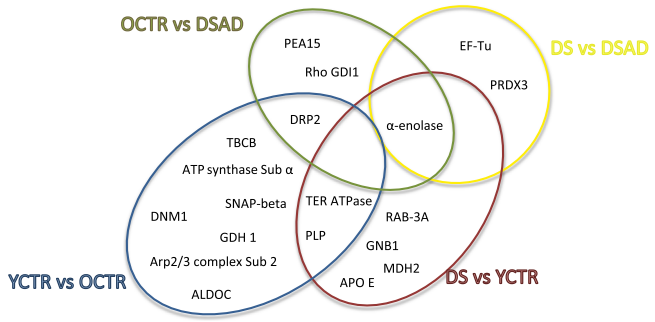

Fig. 2. Venn diagram of overlapping proteins between the four groups of analysis.

antioxidant function, and energy metabolism, respectively, with decreased expression in the DS group with AD neuropathology.

EF-Tu is involved in the protein synthesis machinery. Human mitochondrial EF-Tu is a nuclear-encoded protein involved in the synthesis of proteins that are part of the electron transport chain and ATP synthetase [29]. This study reports a significant decrease in EF-Tu levels in DS frontal cortex when AD neuropathology is present. EF-Tu was also significantly downregulated 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 of DS, transgenic mice expressing wild-type human SOD1, showed significantly decreased hippocampal 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, PRDX3, to be significantly decreased in the 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 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 the 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 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 comparing young controls to young DS and to DS with AD neuropathology. Furthermore, our data were in line with a recent redox proteomics study that revealed that α-enolase was significantly oxidized in the DS group with and without AD neuropathology compared to age-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, as evaluated by positron emission tomography, compared 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 prosurvival 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, MDH was overexpressed in young DS compared to controls. Encoded by the MDH2 gene, MDH is the final enzyme in the mitochondrial tricarboxylic acid cycle. MDH activity is elevated in the brains of subjects with AD [46,47]. This enzyme was also 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 covalently bound by HNE in early AD in the inferior parietal lobule compared to similarly aged control brain [49]. Because 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 protein levels were significantly increased in young DS compared to the other analyzed groups [24]. Furthermore, cytosolic MDH analyzed by redox proteomics in the same samples used in this study also has shown higher levels of oxidation in a DS group with AD neuropathology compared to an 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 APO E in frontal cortex in young DS cases compared to the age-matched control group were observed. APO E is an apolipoprotein that in the brain plays an important role in the regulation of cholesterol and Aβ transport and clearance. APO E plays a role in neuronal plasticity, neurite outgrowth, and synaptogenesis [50]. Studies suggest that APO E-null mice develop mild to severe spatial learning and memory deficits [51,52] and elevated oxidative stress [53]. Furthermore, it was demonstrated that APO E-deficient mice were also more susceptible to neurodegeneration than their wild-type counterparts. Indeed, the deficiency of APO E significantly exacerbates the formation of Aβ-like deposition as an early event before any central nervous system defects [54]. The decreased level of APO E 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 significantly decreased in the frontal cortex from young DS people compared to the age-matched healthy subjects is Rab-3 A. This protein is specifically expressed

in the brain, localized at the presynaptic level, and it regulates the synaptic vesicle exocytosis of neurotransmitters [56]. In a previous work, Reddy et al. [57] have shown a substantial loss of both presynaptic vesicle proteins and postsynaptic proteins, including Rab 3 A, in brains from AD patients compared to age-matched control subjects. Furthermore, they found that the presynaptic proteins synaptophysin and Rab-3 A and the postsynaptic protein synaptopodin were the most downregulated 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 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 have been implicated in the pathogenesis of several neurodegenerative diseases, such as Parkinson disease, 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 overexpressed in the 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 the 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 Fig. 2, α-enolase plays a critical role in DS and in DS/AD 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 DS/AD and are ongoing in our laboratories [23].

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