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Association between frontal cortex oxidative damage and beta-amyloid as a function of age in Down syndrome

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ABSTRACT

Down syndrome (DS) is the most common genetic cause of intellectual disability in children, and the number of adults with DS reaching old age is increasing. By the age of 40 years, virtually all people with DS have sufficient neuropathology for a postmortem diagnosis of Alzheimer disease (AD). Trisomy 21 in DS leads to an overexpression of many proteins, of which at least two are involved in oxidative stress and AD: superoxide dismutase 1 (SOD1) and amyloid precursor protein (APP). In this study, we tested the hypothesis that DS brains with neuropathological hallmarks of AD have more oxidative and nitrosative stress than those with DS but without significant AD pathology, as compared with similarly aged-matched non-DS controls. The frontal cortex was examined in 70 autopsy cases (n = 29 control and n = 41 DS). By ELISA, we quantified soluble and insoluble Aβ40 and Aβ42, as well as oligomers. Oxidative and nitrosative stress levels (protein carbonyls, 4-hydroxy-2-*trans*-nonenal (HNE)-bound proteins, and 3-nitrotyrosine) were measured by slot-blot. We found that soluble and insoluble amyloid beta peptide (Aβ) and oligomers increase as a function of age in DS frontal cortex. Of the oxidative stress markers, HNE-bound proteins were increased overall in DS. Protein carbonyls were correlated with Aβ40 levels. These results suggest that oxidative damage, but not nitrosative stress, may contribute to the onset and progression of AD pathogenesis in DS. Conceivably, treatment with antioxidants may provide a point of intervention to slow pathological alterations in DS.

1. Introduction

One of the most common genetic abnormalities in live-born children in the United States (1 in 700–1000) is Down syndrome (DS) [20]. DS is linked to an extra copy of chromosome 21 [42]. In addition to intellectual disability, children often have cardiac and gastrointestinal congenital malformations, various types of leukemia, growth retardation, immune disorders and other clinical pathologies [66]. A key concern in adults with DS is an increased vulnerability to the development of Alzheimer disease (AD), which typically has an age of onset between 40 and 60 years [71].

A link between DS and AD has been established [4, 12, 47, 48]. Virtually all DS adults over the age of 40 years show neuropathological hallmarks of AD, including senile plaques (SPs) and neurofibrillary tangles (NFTs) [30, 53, 81]. SPs are primarily composed of amyloid beta peptide (Aβ) produced via sequential cleavage of the amyloid precursor protein (APP) by beta- and gamma-secretase [73]. Several peptides of varying lengths are produced, but the most actively studied are the 42 amino acid fragment (Aβ42) and the more soluble 40 amino acid peptide (Aβ40) [72]. Aβ42 shows a higher propensity to adopt neurotoxic conformations, including oligomers [46, 61]. Oligomers of Aβ have been increasingly implicated in the initiation and pathogenesis of AD, while monomeric forms of Aβ may be less harmful [23, 78]. In DS, the accumulation of Aβ42 in brain can be observed as young as between 8 and 12 years of age [43, 45]. The extent of

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SP deposition increases markedly between 35 and 45 years, with NFTs developing after SPs [52, 81]. Deposits of Aβ in DS are first seen in the frontal and entorhinal cortex and spread to other cortical regions and layers with increasing age [3]. Interestingly, the incidence of dementia typically does not increase until adults with DS are over the age of 50 years [39, 48, 69, 75], suggesting a ~10 year prodromal phase where clinical signs are minimal or not detectable.

Similar to AD ([2, 29, 54, 74]; Butterfield et al.), Aβ accumulation in DS is associated with enhanced formation of reactive oxygen species (ROS) in neurons leading to premature neuronal dysfunction and death as a consequence of increased oxidative stress [11, 37, 50]. Interestingly, intracellular Aβ accumulation is observed early in DS, prior to the accumulation of extracellular Aβ deposits [18, 19]. Subsequently high molecular weight aggregates of Aβ may accumulate, enhancing the deposition of plaques [27, 38] and ROS production [6]. Moreover, of many genes overexpressed due to trisomy 21, several are particularly relevant for the development of AD in DS. Among these, APP and cytoplasmic superoxide dismutase (Cu^{2+}/Zn^{2+}; SOD1) play a pivotal role in the regulation of oxidative and nitrosative stress levels [17, 68].

To characterize age- and AD-associated changes in oxidative and nitrosative stress in the frontal cortex from DS autopsy cases, we quantified two markers of protein oxidation (protein carbonyls [PCs] and 3-nitrotyrosine [3-NT]) and a marker of protein modification that is a lipid peroxidation product (4-hydroxy-2-*trans*-nonenal [HNE]). In these samples, we also examined the levels of Aβ40 and Aβ42 and Aβ oligomers. We hypothesized that oxidative damage would be higher overall in DS as compared to non-DS, but further exacerbated with the development of AD neuropathology. In addition, we hypothesized that the extent of oxidative and nitrosative damage would be associated with levels of age-associated Aβ accumulation in DS brain.

2. Materials and methods

2.1. Subjects

DS and young or non-demented older control cases were obtained from the University of California-Irvine-ADRC Brain Tissue Repository, the Eunice Kennedy Shriver NICHD Brain and Tissue Bank for Developmental Disorders, and the University of Kentucky ADC. Table 1 shows the characteristics of the included cases. DS cases were divided into two groups, with or without sufficient pathology for a neuropathology diagnosis of AD. All cases with both DS and 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 post mortem interval (PMI) was different across groups, with the AD group overall having a lower PMI ($F(3,66)=7.3$ $p<0.0005$). A subset of these autopsy cases was used in a previous experiment measuring insoluble Aβ as a function of age in DS [56]. In the current study, additional cases were included, soluble Aβ was measured and the extent of oligomer accumulation was quantified.

Table 1
Case demographics.

Group	n	Gender (M/F)	Age (SEM)	PMI (SEM)
Young control (YC)	13	8/5	17.8 (3.7)	16.2 (1.9)
Old control (OC)	16	7/9	50.8 (2.2)	14.3 (2.0)
DS	13	7/6	27.2 (5.1)	16.0 (1.7)
DS + AD	28	12/16	53.8 (2.5)	6.91 (1.2)

2.2. Sample preparation for oxidative stress measures

Brain tissue (frontal cortex) from non-DS controls, DS, and DS with AD 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 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).

2.3. Measurement of protein carbonyls (PCs)

Five microliters of frontal cortex homogenate were derivatized with 10 μl of 10 mM 2,4-dinitrophenylhydrazine (DNPH) (OxyBlot™ Protein Oxidation Detection Kit, Chemicon-Millipore, Billerica, MA) in the presence of 5 μl of 10% sodium dodecyl sulfate (SDS) for 20 min at room temperature (25 °C). The samples were then neutralized with 7.5 μl of 2 M Tris in 30% glycerol. Protein samples (250 ng) were then loaded in each well on a nitrocellulose membrane with a slot-blot apparatus under vacuum. The membrane was blocked for 2 h with a solution of 3% (w/v) bovine serum albumin in PBS containing 0.01% (w/v) sodium azide and 0.2% (v/v) Tween 20. Membranes were incubated with rabbit polyclonal anti-DNP antibody (1:100 dilution, OxyBlot™ Protein Oxidation Detection Kit) for 2 h at room temperature. After washing with PBS, membranes were further incubated with alkaline phosphatase-conjugated goat anti-rabbit secondary antibody (1:5000; Sigma Aldrich, St. Louis, MO) for 1 h at room temperature. Membranes were then washed with PBS three times for 5 min and developed using 5-bromo-4-chloro-3-indolyl-phosphate/nitroblue tetrazolium (BCIP/NBT) color developing reagent for alkaline phosphatase activity (Sigma Aldrich). Blots were dried and scanned to TIF format using Adobe Photoshop on a Canoscan 8800F (Canon, Lake Success, NY). The images were quantified with Image Quant TL 1D version 7.0 software (GE Healthcare, Fairfield, CT).

2.4. Measurement of protein-bound 4-hydroxy-2-trans-nonenal (HNE-bound protein)

For the analysis of HNE-bound protein levels, 10 μl of frontal cortex homogenate were incubated with 10 μl of Laemmli buffer containing 0.125 M Tris base pH 6.8, 4% (v/v) SDS, and 20% (v/v) glycerol. The resulting samples (250 ng per well) were loaded onto a nitrocellulose membrane with a slot-blot apparatus under vacuum pressure. The membrane was blocked as described above for 2 h and incubated with a rabbit polyclonal anti-4-hydroxynonenal antibody (1:3000; Alpha Diagnostics, San Antonio, TX) for 2 h at room temperature. Membranes were washed and incubated with anti-rabbit IgG alkaline phosphatase secondary antibody (1:5000; Sigma-Aldrich) for 1 h at room temperature. Membranes were then processed and quantified as described above.

2.5. Measurement of 3-nitrotyrosine (3-NT)

3-NT content was determined immunochemically as previously described [16]. Briefly, 5 μl of frontal cortex homogenate were incubated with Laemmli sample buffer in a 1:2 ratio (0.125 M Trizma base, pH 6.8, 4% SDS, 20% glycerol) for 20 min. Protein (250 ng per well) was then loaded onto the nitrocellulose membrane using the slot-blot apparatus as described above. Membranes were incubated with rabbit anti-nitrotyrosine antibody (1:1000; Sigma-Aldrich) for 2 h at room temperature. Membranes were then washed and incubated with alkaline phosphatase-linked anti-rabbit

IgG secondary antibody (1:5000; Sigma-Aldrich) for 1 h at room temperature. Membranes were then processed and quantified as described above.

2.6. Aβ ELISAs

Aβ was extracted from tissue measured as previously described [5]. Briefly, frozen cortical samples were extracted sequentially in ice cold phosphate buffered saline (PBS, pH 7.4) with a complete protease inhibitor cocktail (PIC) (Amresco, Solon, OH) and centrifuged at 20,800×*g* for 30 min. at 4 °C. Following centrifugation, the supernatant was collected and the pellets were sonicated (10×0.5 s pulses at 100 W, Sonic Dismembrator, Fisher Scientific, Pittsburgh, PA) in 2% SDS with PIC and centrifuged at 20,800×*g* for 30 min. at 14 °C. The supernatant was again collected and the remaining pellets were sonicated in 70% formic acid (FA), followed by centrifugation at 20,800×*g* for 1 h at 4 °C.

FA-extracted material was initially neutralized by a 1:20 dilution in TP buffer (1 M Tris base, 0.5 M Na_2HPO_4), followed by a further dilution as needed (1:100 to 1:400) in Antigen Capture buffer (AC) (20 mM Na_3PO_4, 0.4% Block Ace (AbD Serotec), 0.05% NaN_3, 2 mM EDTA, 0.4 M NaCl, 0.2% BSA, 0.05% CHAPS, pH 7). SDS soluble fractions were diluted (1:20) in AC buffer alone. PBS fractions were diluted 1:4 in AC buffer alone.

Aβ was measured in tissue samples using a standard, well-characterized two-site sandwich ELISA as described previously [47]. Briefly, an Immulon 4HBX plate was coated with 0.5 μg antibody per well, incubated overnight at 4 °C, and blocked with a solution of Synblock (AbD Serotec, Raleigh, NC), as per the manufacturer's instructions. Antigen capture was performed using monoclonal antibody Ab9 (against Human Aβ1–16). Antigen detection was performed using biotinylated antibodies 13.1.1 (specific for Aβ40) and 12F4 (specific for Aβ42; Covance, Princeton, NJ).

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

2.7. Oligomer assay

Aβ oligomers from the SDS-soluble fraction were measured using a single-site sandwich ELISA as described above, except the same antibody (4G8; Covance, Princeton, NJ) was used for capture and detection. SDS samples were diluted 1:50 in AC buffer. Synthetic Aβ42 oligomers were used to prepare a standard curve; oligomeric Aβ values were determined by interpolation relative to the standard curve.

2.8. Statistical analysis

A univariate analysis of covariance (ANCOVA) with PMI as the covariate was used to compare groups on different outcome measures reflecting oxidative stress. In these analyses, we compared DS v. non-DS. Further, we placed DS cases into two groups, either having insufficient pathology for a diagnosis of AD or having sufficient neuropathology for a diagnosis of AD. Since the majority of DS cases with AD neuropathology were over 40 years, the control cases were categorized as either ≤40 or >40 years old. For comparison of two groups, independent *t*-tests were used. Spearman rank correlations were calculated to test the association between age and oxidative damage, as well as oxidative damage and Aβ. All statistics were calculated using PASW (IBM, Chicago, IL) and evaluated using a p-value of <0.05.

3. Results

3.1. Effect of PMI and gender on oxidative and nitrosative stress marker levels in the brain from control, DS, and DS with AD subjects

We first determined whether PMI was a significant contributor to the various measures of oxidative damage, given that DS with AD cases overall had shorter PMIs. The correlation between PMI and PCs ($r=-0.34$, $p=0.004$), 3-NT ($r=-0.015$, $p=0.90$) and HNE-bound proteins ($r=-0.10$, $p=0.41$) show that PCs are negatively correlated with PMI. Thus, for subsequent analyses, PMI was included as a covariate. We then examined whether gender ($n=34$ male and $n=35$ female) was a contributor to the outcomes. All samples were combined for this analysis and an independent *t*-test did not show gender effects on PCs ($t(67)=0.58$, $p=0.57$), 3-NT ($t(67)=-0.42$, $p=0.68$), or HNE-bound proteins ($t(67)=0.0.54$, $p=0.59$).

3.2. Oxidative and nitrosative marker levels in control, DS, and DS with AD subjects

We tested the hypothesis that oxidative damage would be higher in DS overall relative to controls and higher still in DS cases with AD neuropathology. An ANCOVA was used with genotype (DS or Control) and age group (≤40 years or >40 years) as factors and PMI as a covariate. No significant differences in PC were noted by genotype ($F(1,69)=0.15$, $p=0.70$), age group ($F(1,69)=0.19$, $p=0.66$), or interaction between genotype and age ($F(1,69)=0.25$ $p=0.62$) (Fig. 1A), although there was a trend towards higher PC in DS cases with AD. Similarly, no significant differences in 3-NT were observed by genotype ($F(1,69)=0.19$ $p=0.7$), age group ($F(1,69)=1.3$ $p=0.3$), or interaction between genotype and age group ($F(1,69)=0.4$ $p=0.5$) (Fig. 1B). Interestingly, HNE-bound proteins were significantly higher overall in DS cases≤40 years of age ($F(1,69)=5.6$ $p=0.02$), but no significant differences were noted by age group ($F(1,69)=0.02$ $p=0.9$) or interaction between age and genotype ($F(1,69)=1.59$ $p=0.2$; Fig. 1C).

3.3. Aβ and oligomer accumulation in DS brain

To determine if soluble or insoluble forms of Aβ as well as oligomers were differentially higher in individuals with DS ± AD relative to controls, a univariate analysis of variance was conducted. For virtually all Aβ outcome measures, there was individual variability, particularly in the older DS brains with significant AD neuropathology. PBS-soluble Aβ40 was significantly higher in individuals>40 years old ($F(1,69)=4.21$ $p=0.044$), but was not significant by genotype ($F(1,69)=2.91$ $p=0.09$) or interaction between age and genotype ($F(1,69)=2.90$ $p=0.093$; Fig. 2A). SDS-extracted Aβ40 was significantly higher in DS ($F(1,69)=4.79$ $p=0.032$) and in cases>40 years old ($F(1,69)=4.83$ $p=0.032$). The interaction between the presence of DS and age>40 years was also significant ($F(1,69)=4.81$ $p=0.032$), with older DS individuals having the highest average amounts of SDS-extracted Aβ40 (Fig. 2B). FA-extracted Aβ40 was significantly higher in individuals with DS ($F(1,69)=3.9$ $p=0.05$) and in individuals>40 years old ($F(1,69)=3.92$ $p=0.05$). Further, as with SDS-extracted Aβ40, FA-extracted Aβ40 was highest in adults with DS over the age of 40 years ($F(1,69)=3.91$ $p=0.05$) (Fig. 2C).

PBS-extracted Aβ42 did not differ across genotype groups or age groups; however, there was significant individual variability (Fig. 2D). Similarly, older individuals had lower PBS Aβ42 than younger individuals ($F(1,67)=3.76$ $p=0.57$), regardless of genotype (Fig. 3D). The interaction between genotype and age group was not significant. SDS-extracted Aβ42 was significantly higher overall in individuals with DS ($F(1,69)=10.89$ $p=0.002$) and in individuals> 40 years old ($F(1,69)=8.4$ $p=0.005$). Further, adults with DS over

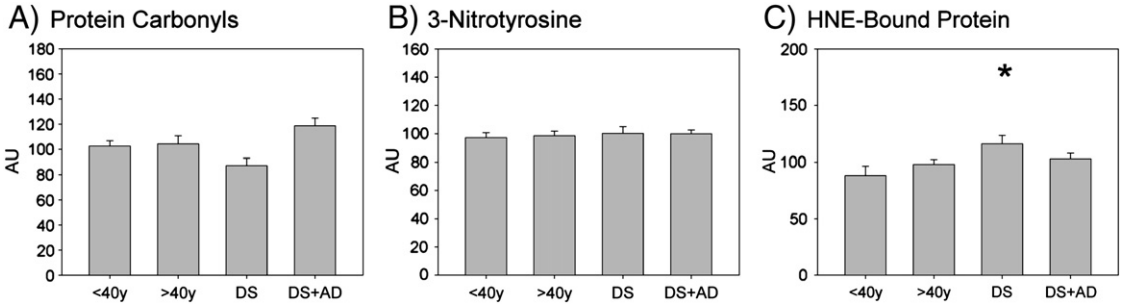

Fig. 1. Levels of oxidative and nitrosative stress markers in control, DS, and DS with AD cases. Protein was extracted from frontal cortex and loaded on nitrocellulose membranes in a slot-blot apparatus. Membranes were probed with either anti-DNP protein adducts polyclonal antibody (Fig. 1A), anti-nitrotyrosine polyclonal antibody (Fig. 1B), or anti-HNE-bound protein polyclonal antibody (Fig. 1C). Data are expressed as mean ± SEM (*p = 0.02).

the age of 40 years had the highest levels of SDS-extracted Aβ42 overall ($F(1,69) = 8.23$ $p = 0.006$; Fig. 2E).

The effect of age on FA-extracted Aβ42 was similar to that of SDS-extracted Aβ42, with cases over 40 years having significantly higher levels of Aβ overall ($F(1,69) = 9.08$ $p = 0.004$). Although DS cases overall had higher levels of FA-extracted Aβ relative to controls, this difference only approached statistical significance due to large individual differences ($F(1,69) = 3.4$ $p = 0.069$) and the interaction between genotype and age was not significant ($F(1,69) = 3.22$ $p = 0.078$) (Fig. 3F). The lack of significance for the interaction term was primarily due to one individual without DS over the age of 40 years with a substantially higher amount of FA-extracted Aβ42 than all the other cases (including DS). When this case was removed, FA-extracted Aβ42 was significantly different by both genotypes ($F(1,68) = 7.9$ $p = 0.006$) and the interaction was also significant ($F(1,68) = 7.58$ $p = 0.008$), indicating that individuals with DS over the age of 40 years had higher levels of FA-extracted Aβ42 overall (Fig. 2F).

As shown in Fig. 4, all measures of Aβ except PBS-extracted Aβ42 were significantly correlated with age in DS cases (Table 2 shows correlation co-efficients). Interestingly, in control cases, SDS-extracted Aβ40 and Aβ42 were both correlated with age (Fig. 3).

Oligomeric Aβ accumulation was not significantly different for genotype ($F(1,69) = 1.64$ $p = 0.21$), although and the main effect of age approached significance ($F(1,69) = 2.96$ $p = 0.09$). The interaction between genotype and age was significant ($F(1,69) = 6.08$ $p = 0.02$), which was a result of individuals with DS over the age of 40 years having significantly higher levels of Aβ oligomers (Fig. 4A). The extent of PBS-extracted Aβ oligomers in DS ($r = 0.37$ $p = 0.018$) but not in control cases was correlated with age (Fig. 4B).

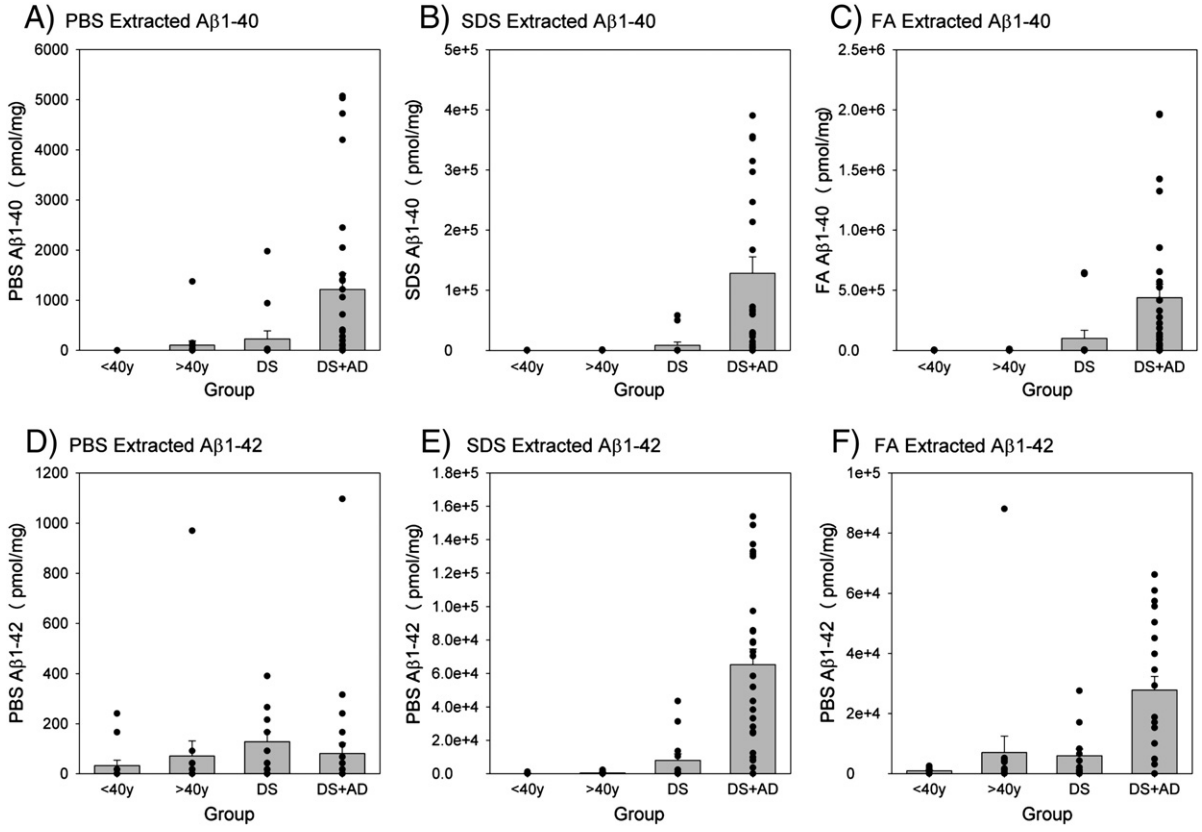

Fig. 2. Soluble and insoluble Aβ40 and Aβ42 as a function of genotype and age group. PBS-, SDS-, and FA-extracted Aβ40 increased with age in both DS and controls (A, B and C). SDS- and FA-extracted Aβ40 were also significantly higher in DS (B and C) and highest in DS with AD. No genotype or age effects were noted for PBS-extracted Aβ42 (D). SDS-extracted Aβ42 was higher overall with age, with DS and DS with AD cases showing the highest levels overall (E). FA-extracted Aβ42 was higher in older cases with or without DS (F). Bars represent mean ± SEM. Closed circles indicate individual data points.

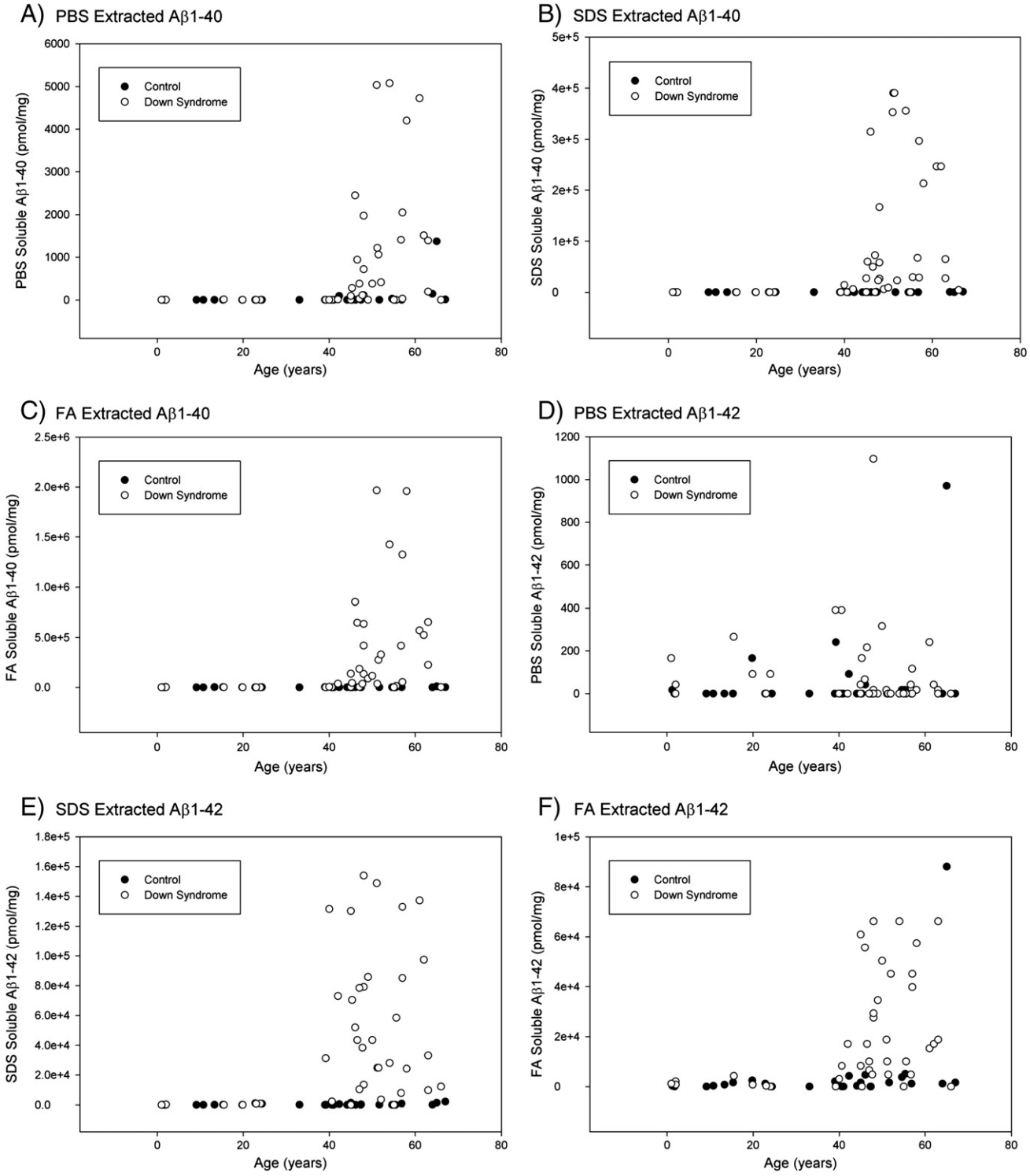

Fig. 3. Correlations between age and Aβ as a function of genotype. PBS-, SDS-, and FA-extracted Aβ40 increased with age in DS (A, B and C). However, in control cases, only SDS-extracted Aβ40 increased with age. SDS- and FA-extracted Aβ40 also increased with age in DS (E and F), although PBS-extracted Aβ42 did not (D). As with SDS-extracted Aβ40, Aβ42 increased with age in control cases, although levels were not as high as in those with DS (E).

3.4. Association between Aβ and oxidative damage in DS

In all Aβ measures, the DS cases and particularly those over the age of 40 years showed significant individual variability. Thus, we hypothesized that individual Aβ measures may reflect differences in the level of oxidative damage. A partial correlation co-efficient that controlled for PMI was calculated between Aβ measures and measures of oxidative damage. The amount of oligomeric Aβ was not correlated with PCs ($r=0.17$ $p=0.16$), NT ($r=-0.07$ $p=0.55$) or HNE ($r=-0.097$ $p=0.43$). Similarly, there were no correlations between any measure of Aβ42 and the extent of PCs (PBS Aβ42 $r=-0.14$ $p=0.27$; SDS Aβ42 $r=0.02$ $p=0.89$; FA Aβ42 $r=0.13$ $p=0.31$), HNE (PBS Aβ42 $r=-0.03$ $p=0.0.83$; SDS Aβ42 $r=0.04$ $p=0.77$; FA Aβ42 $r=-0.10$ $p=0.43$), or 3-NT (PBS Aβ42 $r=-0.18$ $p=0.38$; SDS Aβ42 $r=-0.07$ $p=0.58$; FA Aβ42 $r=-0.14$ $p=0.91$). There was a trend towards PBS-extracted Aβ40 being

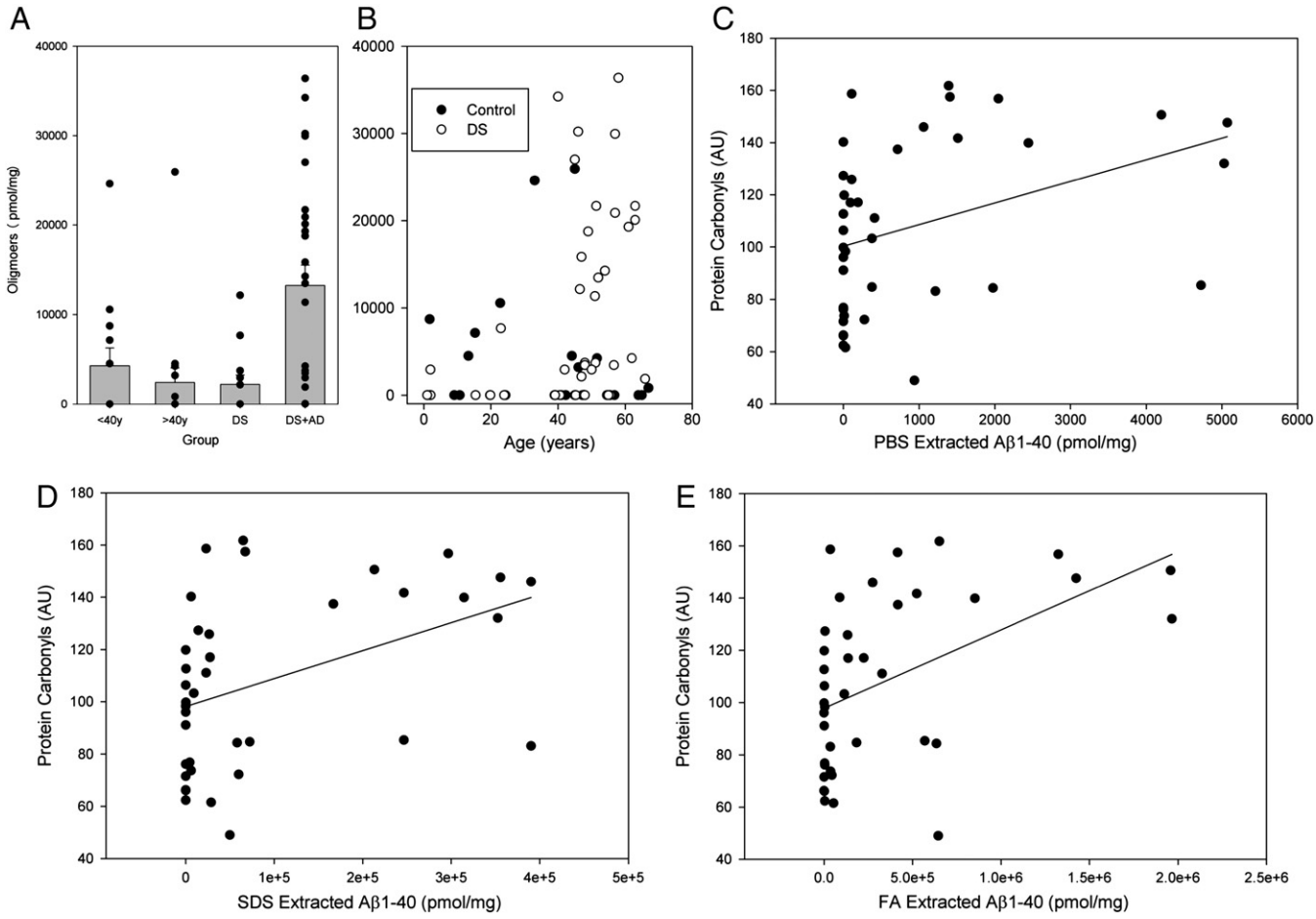

Fig. 4. Aβ Oligomer accumulation with age and DS and correlations between oxidative damage and Aβ. Oligomer accumulation was significantly higher in DS cases over the age of 40 years with significant AD neuropathology (A). Further, oligomers increased as a function of age in DS but not in control cases (B). Higher levels of SDS-extracted Aβ40 (D) and FA-extracted Aβ40 (E) were significantly correlated with higher levels of PCs. A similar trend was observed between PBS-extracted Aβ40 and PCs (C). Bars represent mean ± SEM. Closed circles indicate individual data points.

correlated with PCs, although the level of significance was marginal ($r = 0.310$ $p = 0.058$ $n = 36$) (Fig. 4C). SDS- ($r = 0.369$ $p = 0.023$ $n = 36$) and FA- ($r = 0.39$ $p = 0.016$ $p = 36$) extracted Aβ40 were correlated with significantly higher PC accumulation, but were not correlated with either HNE or 3-NT levels (Fig. 4D, E).

4. Discussion

An imbalance between pro-oxidant stimuli and cellular antioxidant activity may lead to increased oxidative stress levels that may have an important role in the development of AD neuropathology in DS [9, 11, 37]. Involvement of oxidative and nitrosative stress-induced neuronal damage is a well-established feature during the development of AD [14, 17, 74]. In the current study, we provided new evidence of higher levels of oxidative damage in brains from individuals with DS, although measures of oxidative damage were not increased further with AD pathology. The frontal cortex of DS subjects had significantly increased HNE-bound proteins levels, a sensitive marker of lipid peroxidation, compared to non-DS controls. Lipid peroxidation leads to various aldehydic products, with 4-hydroxy-2-nonenal (HNE) being one of the most abundant [76]. HNE is a highly reactive alkenal responsible for the damaging effects of oxidative stress and linked to neurodegenerative diseases such as AD [7, 14, 65].

This study extends earlier studies demonstrating that lipoperoxidation is enhanced in prenatal DS brains compared to non-DS controls [8, 59]. Pratico et al. also demonstrated that another marker of lipid peroxidation, 8,12-iso-iPF2α-VI, was significantly increased in the urine of young subjects with DS, as compared to age-matched controls [63]. Furthermore, cortical neurons cultured from prenatal DS cases exhibited the intracellular accumulation of ROS and increased lipid peroxidation, leading to neuronal apoptosis [11]. A recent study showed that amniotic fluid from mothers carrying DS fetuses had significantly elevated markers of oxidation [60]. HNE-bound proteins were also present in the embryonic brains of Ts1Cje mice, a transgenic animal model of DS [34]. In combination, these data suggest that lipid peroxidation is an early and possibly chronic event in DS.

Table 2
Correlations between Aβ and age in DS and controls.

Genotype	n	PBS extracted Aβ1–40	SDS extracted Aβ1–40	FA extracted Aβ1–40
Control	29	0.32	0.37*	0.22
DS	40	0.37*	0.38*	0.37*
Genotype	**n**	**PBS extracted Aβ1–42**	**SDS extracted Aβ1–42**	**FA extracted Aβ1–42**
Control	29	0.26	0.40*	0.31
DS	40	−0.08	0.37*	0.42*

* $p < .05$.

Increased lipid peroxidation in DS may be a result of the overexpression of one of the key enzymes in the regulation of oxidative stress, SOD1 [21, 24]. Levels of SOD1 in cells from DS patients are approximately 50% greater than normal [25]. In general, SOD1 is responsible for converting superoxide radical into hydrogen peroxide, which is subsequently neutralized by glutathione peroxidase or catalase [32]. If catalase or glutathione peroxidase activity in DS brains is not able to compensate for the increased superoxide dismutase level and activity, hydrogen peroxide may accumulate, leading to increased oxidative damage [35, 63]. Consistent with this hypothesis, gene transfection of SOD1 into two different cell lines leads to increased lipid peroxidation, elevated PCs, and a trend to toward increased levels of 8-hydroxyguanine and 3-NT [41]. However, in the current study, 3-NT levels, a marker of protein nitration and nitrosative stress, was neither higher in DS nor further increased with AD neuropathology. Surprisingly, PCs, a marker of protein oxidation that typically shows robust increases in AD in the general population [2], showed a trend towards increasing levels in DS with AD neuropathology but did not reach statistical significance, most likely due to individual variability in DS cases.

The gene for APP, a key protein involved in AD, is located on chromosome 21 [80]. The overexpression of APP in DS is associated with increased concentrations of Aβ in the brains of these individuals, and Aβ neuropathology has been well characterized in DS [30, 52, 81]. Aβ plaques have been observed as young as 8 years of age and consistently accumulate after 30 years of age [43, 45]. The prevalence of dementia, however, does not increase substantially until after 50 years of age and some individuals with DS remain dementia-free [71, 75]. In an extension of previous work [56], we now show that both soluble and insoluble Aβ40 and Aβ42 in the frontal cortex increase with age in DS. The one exception appears to be a PBS-soluble form of Aβ42 peptide, which did not show a consistent age or genotype association.

We provide novel information regarding oligomer accumulation in DS as a function of age and genotype in a large autopsy cohort. There were no significant group mean differences between cases with or without DS. However, individuals with DS over the age of 40 years with AD neuropathology have significantly higher levels of Aβ oligomers. Further, age and oligomer accumulation were significantly correlated in DS but not controls. These data confirm a previous case report in which immunohistochemistry was performed on the brain of a 46 year old subject with DS and AD, in which plaque-associated oligomer deposits were seen in the entorhinal and frontal cortex [49]. In addition, using an antibody that recognizes Aβ fibrils and soluble fibrillar oligomers, we previously demonstrated that DS individuals show an early age of fibrillar Aβ neuropathology onset in hippocampus and midfrontal gyrus, as compared to aged non-DS individuals [67]. In addition, age-dependent accumulation of Aβ fibrils increased as a function of age in DS, which was absent in non-DS controls [67].

Once Aβ is cleaved from APP, it may appear first as a soluble isoform. Soluble isoforms of Aβ, including oligomers, protofibrils, and Aβ-derived diffusible ligands may initially accumulate inside neurons and may be more important in causing neuronal dysfunction than extracellular Aβ. These different assembly states of soluble Aβ show different toxicities [22, 44]. For example, Aβ oligomers are toxic to neurons both *in vitro* [22, 40] and *in vivo* [44, 78]. Further, oligomeric Aβ may cause mitochondrial dysfunction through interactions with dynamin-related protein 1, exacerbating the oxidative defect in DS [51]. Therefore, Aβ oligomers may be important in causing neuronal dysfunction before neuronal loss occurs during aging in DS [36]. By studying the brains of individuals with DS that came to autopsy at a wide range of ages, it may be possible to determine temporal events associated with soluble and insoluble Aβ, oligomers, and intracellular Aβ that may precede the onset of clinical signs of dementia.

Aβ is associated with oxidative stress *in vitro* and *in vivo* [13, 15, 28]. The presence of Aβ deposits in DS brain may be a critical factor in the generation of oxidative stress, similar to AD patients in the general population [29, 54, 74]. In turn, neuronal oxidative stress may lead to enhanced Aβ production [58, 62]. Indeed, a previous report of oxidized Aβ in the frontal and entorhinal cortex from AD and DS cases showed that this modification was associated with neuritic plaques, suggesting that oxidation of Aβ is an important event in plaque biogenesis [27]. Therefore, increased oxidative damage in DS may affect a variety of pathways.

Oxidative damage may lead to enhanced Aβ production [58, 62, 64] and vice versa, higher levels of Aβ may exacerbate ongoing oxidative damage [6, 31, 77]. In DS, abnormal APP processing may result from increases in reactive oxygen species production due to mitochondrial dysfunction [10]. Increased oxidative damage reflecting overexpression of SOD1 in DS may also impact degradation of Aβ, as enzymes responsible for Aβ degradation and clearance may themselves be vulnerable to oxidative damage. A rise in oxidized neprilysin, an enzyme responsible for degrading Aβ, in AD brain may provide one mechanism underlying the accumulation of extracellular Aβ [79].

The data reported in this study demonstrate a selective positive correlation between one marker of oxidative stress (PCs) and the levels of Aβ40 peptide. Although Aβ42 is generally regarded as the more toxic of the two peptides, Aβ40 has been shown to be neurotoxic in neuronal cultures [1, 26]. Furthermore, Aβ40 may exert neurotoxic properties on neuronal progenitor cells, impairing the survival and differentiation of these cells by generating oxidative damage [55]. Lastly, Aβ40 fibrils are able to directly induce specific nitrosative and oxidative modifications such as oxidation, glycation, and 3-nitrotyrosination in cell proteins [33]. However, it is surprising that we did not see an association between Aβ42 and oxidative damage. Indeed, Aβ42 is capable of causing oxidative stress through free radical reactions [82]. One possible explanation of these data could be related to the amount of Aβ42 and Aβ40 measured in the frontal cortex. The amount of Aβ40 is consistently higher than Aβ42 in the frontal cortex in DS, particularly in DS with AD. Therefore, we hypothesize that the oxidative stress observed in DS brains may be more strongly associated with Aβ40 than Aβ42. Interestingly, increasing plasma Aβ40 levels in adults with DS is associated with onset of dementia [70]. Interestingly, prior to Aβ deposition in DS, oxidized DNA/RNA rises with age but once Aβ42 is deposited in DS temporal cortex there appears to be a decrease in the level of oxidized DNA/RNA [57, 58]. It is possible that Aβ might serve an antioxidant role in DS and is consistent with the lack of further increase in oxidative damage noted in the current study in individuals with both DS and AD. We note, however, that both measures of oxidative damage and Aβ were by immunohistochemical methods whereas the current study used bulk protein measures.

In summary, we demonstrate that oxidative stress, but not nitrosative stress, is present in DS brain. Specifically, markers of oxidative stress are significantly correlated with Aβ40 monomer levels. Lipid peroxidation appears to be an early event occurring in DS individuals, as it is present in both younger and older cases. Increased oxidative stress may leave the DS brain vulnerable to subsequent AD neuropathology. The oxidative and nitrosative protein modifications analyzed in the frontal cortex of DS, DS with AD, and control individuals can lead to dysfunctional proteins. Future experiments should focus on determining which proteins are modified by oxidative stress in DS. We also note that our measures represent bulk determinations of proteins, and not only will neuronal and glial cell populations contribute to these results but importantly, vascular cells will also contribute significantly. Proteomics analysis may be a useful approach for this type of investigation, resulting in a better understanding of which biological mechanisms are involved in the onset and progression of AD in DS, which may then provide useful information for clinical trials in DS.

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References

[1] M.Y. Aksenov, M.V. Aksenova, W.R. Markesbery, D.A. Butterfield, Amyloid beta-peptide (1–40)-mediated oxidative stress in cultured hippocampal neurons. Protein carbonyl formation, CK BB expression, and the level of Cu, Zn, and Mn SOD mRNA, J. Mol. Neurosci. : MN 10 (1998) 181–192.
[2] M.Y. Aksenov, M.V. Aksenova, D.A. Butterfield, J.W. Geddes, W.R. Markesbery, Protein oxidation in the brain in Alzheimer's disease, Neuroscience 103 (2001) 373–383.
[3] B.Y. Azizeh, E. Head, M.A. Ibrahim, R. Torp, A.J. Tenner, R.C. Kim, I.T. Lott, C.W. Cotman, Molecular dating of senile plaques in the brains of individuals with Down syndrome and in aged dogs, Exp. Neurol. 163 (2000) 111–122.
[4] M.J. Ball, K. Nuttall, Neurofibrillary tangles, granulovacuolar degeneration, and neuron loss in Down syndrome: quantitative comparison with Alzheimer dementia, Ann. Neurol. 7 (1980) 462–465.
[5] T.L. Beckett, D.M. Niedowicz, C.M. Studzinski, A.M. Weidner, R.L. Webb, C.J. Holler, R.R. Ahmed, H. LeVine III, M.P. Murphy, Effects of nonsteroidal anti-inflammatory drugs on amyloid-beta pathology in mouse skeletal muscle, Neurobiol. Dis. 39 (2010) 449–456.
[6] C. Behl, J.B. Davis, R. Lesley, D. Schubert, Hydrogen peroxide mediates amyloid beta protein toxicity, Cell 77 (1994) 817–827.
[7] M.A. Bradley, S. Xiong-Fister, W.R. Markesbery, M.A. Lovell, Elevated 4-hydroxyhexenal in Alzheimer's disease (AD) progression, Neurobiol. Aging (2010), doi: 10.1610/j.neurobiolaging.2010.08.016.
[8] B.W. Brooksbank, M. Martinez, R. Balazs, Altered composition of polyunsaturated fatty acyl groups in phosphoglycerides of Down's syndrome fetal brain, J. Neurochem. 44 (1985) 869–874.
[9] J. Busciglio, J.K. Andersen, H.M. Schipper, G.M. Gilad, R. McCarty, F. Marzatico, O. Toussaint, Stress, aging, and neurodegenerative disorders. Molecular mechanisms, Ann. N. Y. Acad. Sci. 851 (1998) 429–443.
[10] J. Busciglio, A. Pelsman, C. Wong, G. Pigino, M. Yuan, H. Mori, B.A. Yankner, Altered metabolism of the amyloid β precursor protein is associated with mitochondrial dysfunction in Down's syndrome, Neuron 33 (2002) 677–688.
[11] J. Busciglio, B.A. Yankner, Apoptosis and increased generation of reactive oxygen species in Down's syndrome neurons in vitro, Nature 378 (1995) 776–779.
[12] A. Bush, N. Beail, Risk factors for dementia in people with down syndrome: issues in assessment and diagnosis, Am. J. Ment. Retard. 109 (2004) 83–97.
[13] D.A. Butterfield, M.L. Bader Lange, R. Sultana, Involvements of the lipid peroxidation product, HNE, in the pathogenesis and progression of Alzheimer's disease, Biochim. Biophys. Acta 1801 (2010) 924–929.
[14] D.A. Butterfield, T. Reed, R. Sultana, Roles of 3-nitrotyrosine- and 4-hydroxynonenal-modified brain proteins in the progression and pathogenesis of Alzheimer's disease, Free. Radic. Res. 45 (2010) 59–72.
[15] D.A. Butterfield, J. Drake, C. Pocernich, A. Castegna, Evidence of oxidative damage in Alzheimer's disease brain: central role for amyloid beta-peptide, Trends Mol. Med. 7 (2001) 548–554.
[16] D.A. Butterfield, T.T. Reed, M. Perluigi, C. De Marco, R. Coccia, J.N. Keller, W.R. Markesbery, R. Sultana, Elevated levels of 3-nitrotyrosine in brain from subjects with amnestic mild cognitive impairment: implications for the role of nitration in the progression of Alzheimer's disease, Brain Res. 1148 (2007) 243–248.
[17] D.A. Butterfield, V. Galvan, M.B. Lange, H. Tang, R.A. Sowell, P. Spilman, J. Fombonne, O. Gorostiza, J. Zhang, R. Sultana, D.E. Bredesen, In vivo oxidative stress in brain of Alzheimer disease transgenic mice: requirement for methionine 35 in amyloid beta-peptide of APP, Free Radic. Biol. Med. 48 (2010) 136–144.
[18] A.M. Cataldo, S. Petanceska, N.B. Terio, C.M. Peterhoff, R. Durham, M. Mercken, P.D. Mehta, J. Buxbaum, V. Haroutunian, R.A. Nixon, Abeta localization in abnormal endosomes: association with earliest Abeta elevations in AD and Down syndrome, Neurobiol. Aging 25 (2004) 1263–1272.
[19] A.M. Cataldo, C.M. Peterhoff, J.C. Troncoso, T. Gomez-Isla, B.T. Hyman, R.A. Nixon, Endocytic pathway abnormalities precede amyloid beta deposition in sporadic Alzheimer's disease and Down syndrome: differential effects of APOE genotype and presenilin mutations, Am. J. Pathol. 157 (2000) 277–286.
[20] CDC, Improved national prevalence estimates for 18 selected major birth defects—United States, 1999–2001, , 2006.
[21] J.B. de Haan, E.J. Wolvetang, F. Cristiano, R. Iannello, C. Bladier, M.J. Kelner, I. Kola, Reactive oxygen species and their contribution to pathology in Down syndrome, Adv. Pharmacol. 38 (1997) 379–402.
[22] A. Deshpande, E. Mina, C. Glabe, J. Busciglio, Different conformations of amyloid beta induce neurotoxicity by distinct mechanisms in human cortical neurons, J. Neurosci. 26 (2006) 6011–6018.
[23] M.L. Giuffrida, F. Caraci, B. Pignataro, S. Cataldo, P. De Bona, V. Bruno, G. Molinaro, G. Pappalardo, A. Messina, A. Palmigiano, D. Garozzo, F. Nicoletti, E. Rizzarelli, A. Copani, Beta-amyloid monomers are neuroprotective, J. Neurosci. : Off. J. Soc. Neurosci. 29 (2009) 10582–10587.
[24] Y. Groner, O. Elroy-Stein, K.B. Avraham, R. Yarom, M. Schickler, H. Knobler, G. Rotman, Down syndrome clinical symptoms are manifested in transfected cells and transgenic mice overexpressing the human Cu/Zn-superoxide dismutase gene, J. Physiol. (Paris) 84 (1990) 53–77.
[25] Y. Groner, O. Elroy-Stein, K.B. Avraham, M. Schickler, H. Knobler, D. Minc-Golomb, O. Bar-Peled, R. Yarom, S. Rotshenker, Cell damage by excess CuZnSOD and Down's syndrome, Biomed. Pharmacother. 48 (1994) 231–240.
[26] M.E. Harris, K. Hensley, D.A. Butterfield, R.A. Leedle, J.M. Carney, Direct evidence of oxidative injury produced by the Alzheimer's beta-amyloid peptide (1–40) in cultured hippocampal neurons, Exp. Neurol. 131 (1995) 193–202.
[27] E. Head, W. Garzon-Rodriguez, J.K. Johnson, I.T. Lott, C.W. Cotman, C. Glabe, Oxidation of Abeta and plaque biogenesis in Alzheimer's disease and Down syndrome, Neurobiol. Dis. 8 (2001) 792–806.
[28] K. Hensley, J.M. Carney, M.P. Mattson, M. Aksenova, M. Harris, J.F. Wu, R.A. Floyd, D.A. Butterfield, A model for beta-amyloid aggregation and neurotoxicity based on free radical generation by the peptide: relevance to Alzheimer disease, Proc. Natl. Acad. Sci. U. S. A. 91 (1994) 3270–3274.
[29] K. Hensley, N. Hall, R. Subramaniam, P. Cole, M. Harris, M. Aksenov, M. Aksenova, S.P. Gabbita, J.F. Wu, J.M. Carney, et al., Brain regional correspondence between Alzheimer's disease histopathology and biomarkers of protein oxidation, J. Neurochem. 65 (1995) 2146–2156.
[30] P.R. Hof, C. Bouras, D.P. Perl, D.L. Sparks, N. Mehta, J.H. Morrison, Age-related distribution of neuropathologic changes in the cerebral cortex of patients with Down's syndrome, Arch. Neurol. 52 (1995) 379–391.
[31] X. Huang, C.S. Atwood, M.A. Hartshorn, G. Multhaup, L.E. Goldstein, R.C. Scarpa, M.P. Cuajungco, D.N. Gray, J. Lim, R.D. Moir, R.E. Tanzi, A.I. Bush, The A beta peptide of Alzheimer's disease directly produces hydrogen peroxide through metal ion reduction, Biochemistry 38 (1999) 7609–7616.
[32] R.C. Iannello, P.J. Crack, J.B. de Haan, I. Kola, Oxidative stress and neural dysfunction in Down syndrome, J. Neural Transm. 57 (1999) 257–267.
[33] G. Ill-Raga, E. Ramos-Fernandez, F.X. Guix, M. Tajes, M. Bosch-Morato, E. Palomer, J. Godoy, S. Belmar, W. Cerpa, J.W. Simpkins, Nc Inestrosa, F.J. Munoz, Amyloid-beta peptide fibrils induce nitro-oxidative stress in neuronal cells, J. Alzheimer's Dis. : JAD 22 (2010) 641–652.
[34] K. Ishihara, K. Amano, E. Takaki, A.S. Ebrahim, A. Shimohata, N. Shibazaki, I. Inoue, M. Takaki, Y. Ueda, H. Sago, C.J. Epstein, K. Yamakawa, Increased lipid peroxidation in Down's syndrome mouse models, J. Neurochem. 110 (2009) 1965–1976.
[35] S.V. Jovanovic, D. Clements, K. MacLeod, Biomarkers of oxidative stress are significantly elevated in Down syndrome, Free Radic. Biol. Med. 25 (1998) 1044–1048.
[36] A.S. Karlsen, B. Pakkenberg, Total numbers of neurons and glial cells in cortex and basal ganglia of aged brains with Down syndrome—a stereological study, Cereb. Cortex 21 (2011) 2519–2524.
[37] J. Kedziora, G. Bartosz, Down's syndrome: a pathology involving the lack of balance of reactive oxygen species, Free Radic. Biol. Med. 4 (1988) 317–330.
[38] M.F. Knauer, B. Soreghan, D. Burdick, J. Kosmoski, C.G. Glabe, Intracellular accumulation and resistance to degradation of the Alzheimer amyloid A4/beta protein, Proc. Natl. Acad. Sci. U. S. A. 89 (1992) 7437–7441.
[39] F. Lai, M.D. Williams, A prospective study of Alzheimer disease in Down syndrome, Arch. Neurol. 46 (1989) 849–853.
[40] M.P. Lambert, A.K. Barlow, B.A. Chromy, C. Edwards, R. Freed, M. Liosatos, T.E. Morgan, I. Rozovsky, B. Trommer, K.L. Viola, P. Wals, C. Zhang, C.E. Finch, G.A. Krafft, W.L. Klein, Diffusable, nonfibrillar ligands derived from Ab1–42 are potent central nervous system neurotoxins, PNAS 95 (1998) 6448–6453.
[41] M. Lee, D. Hyun, P. Jenner, B. Halliwell, Effect of overexpression of wild-type and mutant Cu/Zn-superoxide dismutases on oxidative damage and antioxidant defences: relevance to Down's syndrome and familial amyotrophic lateral sclerosis, J. Neurochem. 76 (2001) 957–965.
[42] J. Lejeune, M. Gautier, R. Turpin, Etude des chromosomes somatiques de neuf enfants mongoliens, C. R. Hebd. Seances Acad. Sci. 248 (1959) 1721–1722.
[43] C.A. Lemere, J.K. Blusztajn, H. Yamaguchi, T. Wisniewski, T.C. Saido, D.J. Selkoe, Sequence of deposition of heterogeneous amyloid beta-peptides and APOE in Down syndrome: implications for initial events in amyloid plaque formation, Neurobiol. Dis. 3 (1996) 16–32.
[44] S. Lesne, M.T. Koh, L. Kotilinek, R. Kayed, C.G. Glabe, A. Yang, M. Gallagher, K.H. Ashe, A specific amyloid-beta protein assembly in the brain impairs memory, Nature 440 (2006) 352–357.
[45] J.B. Leverenz, M.A. Raskind, Early amyloid deposition in the medial temporal lobe of young Down syndrome patients: a regional quantitative analysis, Exp. Neurol. 150 (1998) 296–304.
[46] S. Li, S. Hong, N.E. Shepardson, D.M. Walsh, G.M. Shankar, D. Selkoe, Soluble oligomers of amyloid beta protein facilitate hippocampal long-term depression by disrupting neuronal glutamate uptake, Neuron 62 (2009) 788–801.
[47] I.T. Lott, Down's syndrome, aging, and Alzheimer's disease: a clinical review, Ann. N. Y. Acad. Sci. 396 (1982) 15–27.
[48] I.T. Lott, E. Head, Down syndrome and Alzheimer's disease: a link between development and aging, Ment. Retard. Dev. Disabil. Res. Rev. 7 (2001) 172–178.
[49] I.T. Lott, E. Head, Alzheimer disease and Down syndrome: factors in pathogenesis, Neurobiol. Aging 26 (2005) 383–389.
[50] I.T. Lott, E. Head, E. Doran, J. Busciglio, Beta-amyloid, oxidative stress and Down syndrome, Curr. Alzheimer Res. 3 (2006) 521–528.
[51] M. Manczak, M.J. Calkins, P.H. Reddy, Impaired mitochondrial dynamics and abnormal interaction of amyloid beta with mitochondrial protein Drp1 in neurons

from patients with Alzheimer's disease: implications for neuronal damage, Hum. Mol. Genet. 20 (2011) 2495–2509.
[52] D.M. Mann, B. Marcyniuk, P.O. Yates, D. Neary, J.S. Snowden, The progression of the pathological changes of Alzheimer's disease in frontal and temporal neocortex examined both at biopsy and at autopsy, Neuropathol. Appl. Neurobiol. 14 (1988) 177–195.
[53] D.M.A. Mann, M.M. Esiri, 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. 89 (1989) 169–179.
[54] W.R. Markesbery, Oxidative stress hypothesis in Alzheimer's disease, Free Radic. Biol. Med. 23 (1997) 134–147.
[55] B. Mazur-Kolecka, A. Golabek, K. Nowicki, M. Flory, J. Frackowiak, Amyloid-beta impairs development of neuronal progenitor cells by oxidative mechanisms, Neurobiol. Aging 27 (2006) 1181–1192.
[56] M. Nistor, M. Don, M. Parekh, F. Sarsoza, M. Goodus, G.E. Lopez, C. Kawas, J. Leverenz, E. Doran, I.T. Lott, M. Hill, E. Head, Alpha- and beta-secretase activity as a function of age and beta-amyloid in Down syndrome and normal brain, Neurobiol. Aging 28 (2007) 1493–1506.
[57] A. Nunomura, G. Perry, K. Hirai, G. Aliev, A. Takeda, S. Chiba, M.A. Smith, Neuronal RNA oxidation in Alzheimer's disease and Down's syndrome, Annals NYAS 893 (1999) 362–364.
[58] A. Nunomura, G. Perry, M. Pappolla, R.P. Friedland, K. Hirai, S. Chiba, M.A. Smith, Neuronal oxidative stress precedes amyloid-β deposition in Down syndrome, J. Neuropathol. Exp. Neurol. 59 (2000) 1011–1017.
[59] P. Odetti, G. Angelini, D. Dapino, D. Zaccheo, S. Garibaldi, F. Dagna-Bricarelli, G. Piombo, G. Perry, M. Smith, N. Traverso, M. Tabaton, Early glycoxidation damage in brains from Down's syndrome, Biochem. Biophys. Res. Commun. 243 (1998) 849–851.
[60] M. Perluigi, F. Di Domenico, A. Fiorini, A. Cocciolo, A. Giorgi, C. Foppoli, D.A. Butterfield, M. Giorlandino, C. Giorlandino, M.E. Schinina, R. Coccia, Oxidative stress occurs early in Down syndrome pregnancy: a redox proteomics analysis of amniotic fluid, Proteomics Clin. Appl. 5 (2011) 167–178.
[61] C.J. Pike, A.J. Walencewicz, C.G. Glabe, C.W. Cotman, In vitro aging of beta-amyloid protein causes peptide aggregation and neurotoxicity, Brain Res. 563 (1991) 311–314.
[62] D. Pratico, K. Uryu, S. Leight, J.Q. Trojanoswki, V.M. Lee, Increased lipid peroxidation precedes amyloid plaque formation in an animal model of Alzheimer amyloidosis, J. Neurosci. 21 (2001) 4183–4187.
[63] D. Pratico, L. Iuliano, G. Amerio, L.X. Tang, J. Rokach, G. Sabatino, F. Violi, Down's syndrome is associated with increased 8,12-iso-iPF2alpha-VI levels: evidence for enhanced lipid peroxidation in vivo, Ann. Neurol. 48 (2000) 795–798.
[64] P.H. Reddy, Amyloid precursor protein-mediated free radicals and oxidative damage: implications for the development and progression of Alzheimer's disease, J. Neurochem. 96 (2006) 1–13.
[65] Y. Riahi, G. Cohen, O. Shamni, S. Sasson, Signaling and cytotoxic functions of 4-hydroxyalkenals, Am. J. Physiol. Endocrinol. Metab. 299 (2010) E879–886.
[66] N.J. Roizen, D. Patterson, Down's syndrome, Lancet 361 (2003) 1281–1289.
[67] F. Sarsoza, T. Saing, R. Kayed, R. Dahlin, M. Dick, C. Broadwater-Hollifield, S. Mobley, I. Lott, E. Doran, D. Gillen, C. Anderson-Bergman, D.H. Cribbs, C. Glabe, E. Head, A fibril-specific, conformation-dependent antibody recognizes a subset of Abeta plaques in Alzheimer disease, Down syndrome and Tg2576 transgenic mouse brain, Acta Neuropathol. 118 (2009) 505–517.
[68] S. Schuchmann, U. Heinemann, Increased mitochondrial superoxide generation in neurons from trisomy 16 mice: a model of Down's syndrome, Free Radic. Biol. Med. 28 (2000) 235–250.
[69] N. Schupf, B. Patel, D. Pang, W.B. Zigman, W. Silverman, P.D. Mehta, R. Mayeux, Elevated plasma beta-amyloid peptide Abeta (42) levels, incident dementia, and mortality in Down syndrome, Arch. Neurol. 64 (2007) 1007–1013.
[70] N. Schupf, W.B. Zigman, M.X. Tang, D. Pang, R. Mayeux, P. Mehta, W. Silverman, Change in plasma Aβ peptides and onset of dementia in adults with Down syndrome, Neurology 75 (2010) 1639–1644.
[71] N. Schupf, G.H. Sergievsky, Genetic and host factors for dementia in Down's syndrome, Br. J. Psychiatry 180 (2002) 405–410.
[72] D.J. Selkoe, Normal and abnormal biology of the beta-amyloid precursor protein, Annu. Rev. Neurosci. 17 (1994) 489–517.
[73] M. Shoji, T.E. Golde, J. Ghiso, T.T. Cheung, S. Estus, L.M. Shaffer, X.D. Cai, D.M. McKay, R. Tintner, B. Frangione, et al., Production of the Alzheimer amyloid beta protein by normal proteolytic processing, Science 258 (1992) 126–129.
[74] M.A. Smith, G. Perry, P.L. Richey, L.M. Sayre, V.E. Anderson, M.F. Beal, N. Kowall, Oxidative damage in Alzheimer's, Nature 382 (1996) 120–121.
[75] J. Tyrrell, M. Cosgrave, M. McCarron, J. McPherson, J. Calvert, A. Kelly, M. McLaughlin, M. Gill, B.A. Lawlor, Dementia in people with Down's syndrome, Int. J. Geriatr. Psychiatry 16 (2001) 1168–1174.
[76] K. Uchida, 4-Hydroxy-2-nonenal: a product and mediator of oxidative stress, Prog. Lipid Res. 42 (2003) 318–343.
[77] S. Varadarajan, S. Yatin, M. Aksenova, D.A. Butterfield, Review: Alzheimer's amyloid β-peptide-associated free radical oxidative stress and neurotoxicity, J. Struct. Biol. 130 (2000) 184–208.
[78] D.M. Walsh, I. Klyubin, J.V. Fadeeva, W.K. Cullen, R. Anwyl, M.S. Wolfe, M.J. Rowan, D.J. Selkoe, Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo, Nature 416 (2002) 535–539.
[79] D.-S. Wang, N. Iwata, E. Hama, T.C. Saido, D.W. Dickson, Oxidized neprilysin in aging and Alzheimer's disease brains, Biochem. Biophys. Res. Commun. 310 (2003) 236–241.
[80] A. Weidemann, G. Konig, D. Bunke, P. Fischer, J.M. Salbaum, C.L. Masters, K. Beyreuther, Identification, biogenesis, and localization of precursors of Alzheimer's disease A4 amyloid protein, Cell 57 (1989) 115–126.
[81] K. Wisniewski, H. Wisniewski, G. Wen, Occurrence of neuropathological changes and dementia of Alzheimer's disease in Down's syndrome, Ann. Neurol. 17 (1985) 278–282.
[82] S.M. Yatin, S. Varadarajan, C.D. Link, D.A. Butterfield, In vitro and in vivo oxidative stress associated with Alzheimer's amyloid beta-peptide (1–42), Neurobiol. Aging 20 (1999) 325–330 (discussion 339–342).