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Original Article

Early age-related progression of AD-like neuropathology in Down's syndrome

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Abstract: We have previously reported that increased numbers of Alz-50-reactive (apoptotic) neurons occurred in young DS subjects compared to controls, but increased in density with increasing age, and in advance of identifiable senile plaques (SP) in DS. The purpose of the study was to determine if there are further differences in Alzheimer's disease (AD)-like neuropathology with increasing age among individuals with Down's syndrome (DS) compared to cognitively normal age-matched controls. The two populations compared were age-matched normal controls (N = 14) between 11 months and 61 years of age and individuals with DS (N = 8) between 1 and 54 years of age. There were 7 cognitively intact DS and 10 control subjects under 35 years of age. The single demented 54 year old DS subject was compared to 4 non-demented controls between 48 and 61 years of age. 50 μm Vibratome sections of formalin fixed hippocampal formations were immunohistochemically stained for amyloid-β (6E10), APP (22C11) and phosphorylated tau (AT8) using standard methods. AT8 immunoreactive features were found only in the oldest DS subject. In contrast, the number and intensity of amyloid-β-immunoreactive neurons were maximal in the youngest DS subjects (1-24 years), reduced in the young adults (25-35 years) synchronous with the appearance of only diffuse-form SP, and were further reduced in the 54 year-old DS subject exhibiting abundant multiform SP. Distribution of APP immunoreactivity (22C11) was distinct from amyloid-β (6E10) in appearance and by location and age in both DS and normal controls. The data indicates that the earliest observable neuropathologic feature in DS may be neuronal accumulation of amyloid-β. Such accumulation of amyloid-β occurs decades in advance of deposition as SP, which in turn occurs decades before cognitive decline.

Keywords: Amyloid-β, amyloid-β precursor protein, phospho-tau

Introduction

End-stage neuropathology in Down's syndrome (DS) is strikingly similar to Alzheimer's disease (AD), in that it culminates in profound dementia and an abundance of senile plaques (SP) and neurofibrillary tangles (NFT) in the brain. DS is for the most part identifiable shortly after birth. Accordinglythis inspired numerous cross-sectional age-related DS investigations of changing cognition and increasingly severe AD-like neuropathology in DS to track the disorder's progression and by association possibly model the progression of AD.

Among the oldest DS individuals it is reported that over three-quarters are profoundly demented at demise [1]. One longitudinal study indicated a 4% prevalence rate among DS subjects in their thirties, 10% in their forties and 40% in their fifties [2]. Another longitudinal study reported a 6% rate in the third decade, increasing to 12% in the fourth decade and 30%in the fifth decade of DS [3].

In our study of 16 DS subjects between 6 and 74 years of age (overlap for the 6, 19 and 54 year old individuals in the current study) it was noted that abundant NFT were found among the 6 individuals over 40 years, all of whom were profoundly demented [4], and essentially absent in the non-demented subjects between 6 and 40 years of age (the occasional NFT was identified in the 35 year old non-demented DS

Table 1. Anamnestic and study data for the Down's syndrome population investigated

case #	Dx	race	age	Cause of death	6E10 #	6E10 intensity	AT8	APP score
1	DS	W	1	Fulminant epiglottitis	245 ± 50	4	Negative	0
2	DS	W	3	Bronchopneumonia	229 ± 39	4	Negative	1
3	DS	W	4	drowning	244 ± 27	5	Negative	0
4	DS	W	19	Sudden cardiac death 2 yrs after cardiac repair	292 ± 24	5	Negative	2
5	DS	W	24	Bronchiolitis	271 ± 36	5	Negative	3
6	DS	W	27	Congenital heart disease - Cushion defect	153 ± 11	3	Negative	3.5
7	DS	W	29	Pulmonarythromboemboli	146 ± 13	3	Negative	3
8	DS	W	54	Blunt force trauma	55 ± 25	1	dense	5

Table 2. Anamnestic and study data for the control population investigated

Case #	Dx	race	age	Cause of death	6e10 #	6E10 intensity	AT8	APP score
1	N	W	11 month	Bronchiolitis	65 ± 40	1	Negative	0
2	N	W	2	Blunt force trauma	28 ± 9	1	Negative	0
3	N	W	6	asphyxia	30 ± 11	1	Negative	0
4	N	W	13	Seizure disorder	91 ± 36	1	Negative	0
5	N	W	15	Chronic renal disease	47 + 18	1	Negative	1
6	N	W	20	Cardiac dysrhythmia	75 + 21	2	Negative	0.5
7	N	W	27	Drug intoxication	49 + 12	1	Negative	0.5
8	N	W	28	overdose	30 + 7	2	Negative	1
9	N	W	30	Myocarditis anesthesia complications	33 + 13	1	Negative	2
10	N	W	33	Gunshot wounds	93 + 21	2	Negative	2
11	N	W	48	Coronary artery disease/Hypertension	96 + 25	2	Negative	3.5
12	N	W	54	Acute myocardial infarction	144 + 27	3	Negative	3
13	N	W	59	overdose	185 + 40	4	Negative	2
14	N	W	61	Coronary artery disease/COPD	114 + 19	3	Negative	3

subject). In this same population abundant SP were apparent among all DS subjects 20 and over, and in no individual under 20 years of age. Based on this all demented DS subject retained SP as did half of the non-demented individuals with DS, leading to the hypothesis that dementia in DS is more closely associated with the presence of NFT rather than SP [4].

An early study of 22 DS subjects between 13 and 64 years of age [5] noted that neither SP or NFT were identified in the two youngest subjects (13 and 31 years of age). It was further revealed that there was no significant difference in the number of hippocampal SP between young (< 50) and old (50 +) non-demented DS subject, but there was a three-fold increase in the number of NFT. It was also reported that a significant 2-fold increase in the number of SP and 5-fold increase in the number of NFT occurred in demented DS subjects compared to non-demented individuals with DS [5].

Another age-related study in 15 DS individuals between 25 and 58 years of age noted that 4 of 4 demented subjects retained NFT but only 4 of the 10 non-demented DS subjects showed NFT, whereas all individuals had varying levels of SP [1]. Plaques in this study were staged [1], in that earlier stages of SP were prominent under 40 years of age while later stage SP were more prevalent in the older DS subjects. Extracellular amyloid-β deposition and neuritic changes without paired helical filaments (PHF) occurred predominantly in the DS individuals between 25 and 38. Dense core SP and PHF containing neurites and "clouds of silver-positive debris" comprised the norm among DS subjects over 48 years of age [1]. Similarly, in a series of 9 adult individuals with DS (41-67

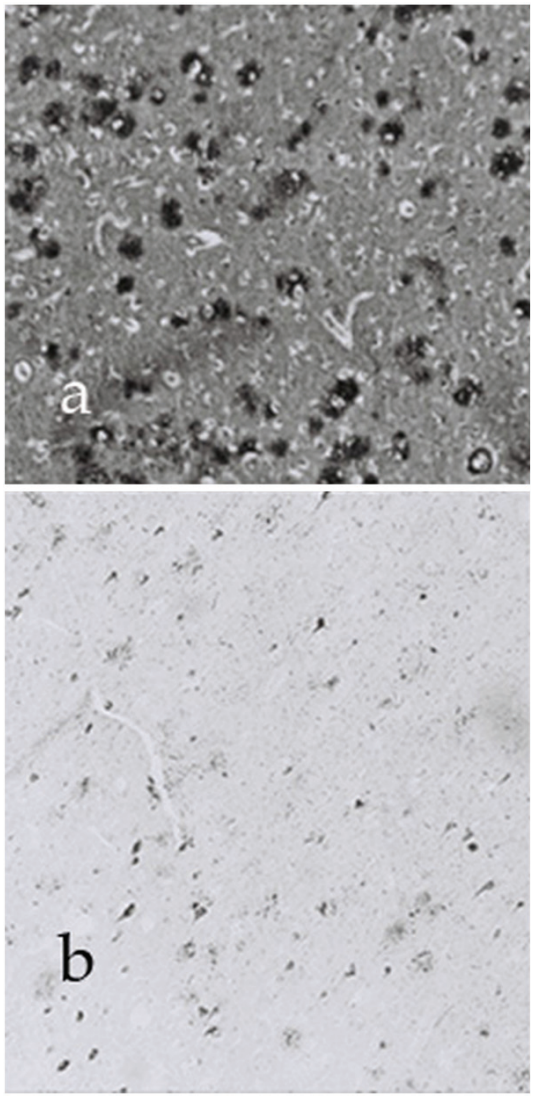

Figure 1. Neuropathology in a demented individual with Down's syndrome. A section of hippocampal formation from a demented 54 year old Down's syndrome subject previously stained by the Bielschowsky method (A) is presented. Similar sections were immunoreacted in the current study with AT8 antibody for phospho-tau (B). All sections were photographed at 10 X.

years of age) diffuse form SP were reported to be the predominant feature in the younger patients while the number of neuritic SP increased with increasing age as numbers of diffuse form SP decreased [6].

Based on the premise that what might occur early in DS may be more closely related to the actual etiology of neuropathology leading to cognitive impairment, we have focused on age-related changes in very young individuals with DS. In a sub-set of the individuals investigated in the current study, we found that increased numbers of Alz-50-reactive (apoptotic; [7]) neurons occurred in adolescent DS subjects compared to controls, but increased in density with increasing age, and the presence of Alz-50-reactive neurons occurred in advance of identifiable SP in DSover 20 years of age [8]. In a separate study, we reported that the appearance of microglial nodules (microgliosis) coincides with the occurrence of senile plaques in DS, but subsequent to evidence of apoptosis [9].

In the current study we investigated 8 DS individuals between 1 and 54 years of age to more fully illuminate the early progression of neuropathology in DS.

Materials and methods

Two groups were compared; individuals with DS (N = 8) between 1 and 54 years of age (**Table 1**) and age-matched cognitively normal controls (N = 14) between 11 months and 61 years of age (**Table 2**). Portions of brain routinely saved as part of a medicolegal death investigation between 1985 and 1999 were assessed microscopically. Postmortem interval was less than 24 hours in all cases. 50μm Vibratome sections of formalin-fixed hippocampal formations were immunohistochemically stained for amyloid-β (6E10; CoVance; 1:250 dilution) and phosphorylated tau (AT8; Pierce Biotechnology; 1:1000 dilution) using standard methods. As 6E10 has been shown to be cross-reactive with amyloid-bprecursor protein (APP), sectionswere also stained using 22C11 antibody (EMD Millipore; 1:200 dilution) directed against full-length APP [10]. Tissue samples from three demented AD subjects were also reacted with 22C11 antibody for comparison to DS and NC subjects (48, 60 and 81 years of age). There were 3 DS and 3 control subjects under 10 years, 1DS subject and 3 controls between 10 and 20 years, and 3 DS and 3 control subjects between 21 and 35 years. The single 54-year-old DS subject was compared to 4 controls between 48 and 61 years of age. Only the 54 year old DS subject was known to be cognitively impaired. Anamnestic data for each individual are summarized in **Tables 1** and **2**.

All stained sections were viewed for neuropathologic features at magnification of 4 and

Figure 2. Neuronal amyloid-β in young Down's and control individuals. Fifty micron Vibratome sections from each DS subject and 14 age-matched non-heart disease normal controls were immunoreacted with 6E10 to localize any amyloid-β staining. The number of amyloid-β immunoreactive neurons is clearly increased in the youngest DS subjects compared to control, and decrease in number and staining intensity in older individuals with DS, although still increased compared to control.

10 X, and photographed at 10 X. The limited nature of AT8 immunoreactivity required that only one stained section (54 yr DS) had to be photographed. The numbers of 6E10 immunoreactive neurons were counted in three random 10 X fields of cortex using a 10 X 10 grid (1 mm^2). Mean number of 6E10 neurons are reported plus/minus the range (high value - low value/2; **Tables 1** and **2**). The intensity of 6E10 immunoreactivity was scored between 0 and + 5 (**Tables 1** and **2**). The features of APP immunoreactivity stained by 22C11 were different from that found for 6E10. Thus different methods of quantification were employed; we used semi-quantification scores of staining in the neuropil and the strip of intensely stained APP immunoreactive spheroids at the interface of the upper cortical layer and the pia was photographed in 10 X fields. Sections from the three AD patients stained for APP were similarly assessed.

Statistics

Mean number of neurons for subjects under the age of 35 were compared between the Down's and control groups using a two sample t-test with unequal variance. Statistical significance was determined at the 0.05 level. In addition, the mean number of neurons was regressed on patient's age and Pearson's linear correlation was computed to assess the fit of the resulting line.

Results

AT8 immunoreactive features were found only in the oldest DS subject (**Figure 1B**). These features retained the appearance of SP like structures in the neuropil, consistent with SP stained by the Bielschowsky silver methods in the same individual (**Figure 1A**). Numerous neurons were identified by AT8 immuno-staining (**Figure 1B**).

In contrast to AT8 staining, the number and intensity of Aβ-immunoreactive neurons were maximal in the youngest DS subjects (1-24 years; **Figure 2**), reduced in the young adults (25-35 years; **Figures 2** and **3**) synchronous with the appearance of only diffuse-form SP stained by 6E10 (not shown), and were further reduced in the 54-year-old DS subject exhibiting abundant multiform SP. The mean Aβ-immunoreactive neuron level was significantly higher ($P < 0.0001$) in the Down's group under 35 years of age (225.7 ± 56.0) than in the age-matched controls (54.1 ± 25.4). The age-related decrease in the number of Aβ-immunoreactive neurons in DS was significant ($p = 0.0257$; **Figure 3**), and a similar age-related decrease in the intensity of staining was also apparent (**Table 1**). In direct opposition to the pattern in DS, we identified a significant age-related increase in the number of Aβ-immunoreactive neurons ($p = 0.0018$; **Figure 4**) and an apparent age-related increase in staining intensity in controls over 50 years of age (**Table 2**).

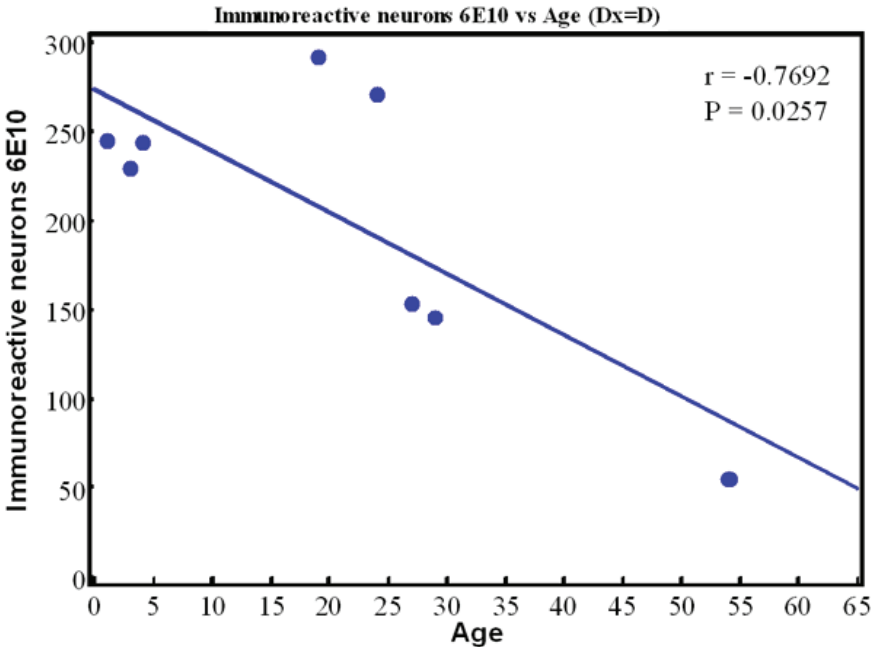

Figure 3. Mean number of amyloid-β immunoreactive neurons for each DS subject plotted according to the individual's age. Sections of hippocampal formation were immunostained with 6E10 using standard methods. The numbers of 6E10 immunoreactive neurons were counted in three random 10 X fields of cortex using a 10 X 10 grid (1 mm^2). The mean number of neurons was regressed on patient's age and Pearson's linear correlation was computed to assess the fit of the resulting line.

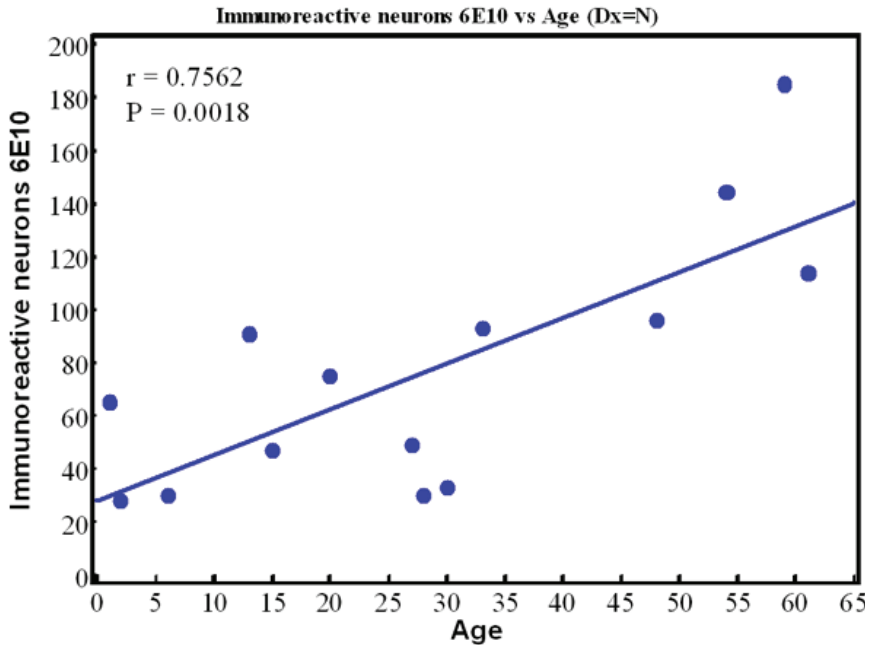

Figure 4. Mean number of amyloid-β immunoreactive neurons for each normal control subject plotted according to the individual's age. Sections of hippocampal formation were immunostained with 6E10 using standard methods. The numbers of 6E10 immunoreactive neurons were counted in three random 10 X fields of cortex using a 10 X 10 grid (1 mm^2). The mean number of neurons was regressed on patient's age and Pearson's linear correlation was computed to assess the fit of the resulting line.

In contrast to findings in transgenic mice [10], the staining of APP with 22C11 did not parallel 6E10 immunoreactive features in DS. Plaque-like structures were APP immunoreactive in the oldest DS subject, but similar features were not identified in any of the 3 AD subjects stained with 22C11 antibody (**Figure 7**). Rather than retaining a neuronalappearance, APP-immunoreactivity presented as 4-10 micron diameter spheres in AD, DS and normal controls (**Figures 5-7**). These spheres were prominent in a ribbon inthe upper pial layer of the cortex, which extended, in a diffuse distribution, into layers II-VI with increasing age in both DS and normal controls (**Figures 5-7**). A similar distribution was found in each of the AD patients, but with a narrower ribbon and somewhat less severe in layers II-VI than the older DS subjects (**Figure 6**). In young controls, APP-immunoreactive spheres occurred only in the pial inter-

Figure 5. APP immunostaining in adolescent DS and normal control individuals. Sections of hippocampal formation were immunostained with 22C11 using standard methods. Montages of the cortical thickness at 10 X magnification is shown for the 3 year old DS subject (left) and the 2 year old normal control (right). The pial surface is at the top of each montage.

face of the cortex (**Figure 5** right), and an obvious increased number of pial spheres occurred in younger DS subjects which were also apparent in upper cortical layers (**Figures 5** and **6**). The density of APP-immunoreactive spheres increased with age in DS (comparing **Figures 5**-**7**; **Table 3**), and in normal controls in the pial surface and lower layers of cortex (**Figures 5**-**7**; **Table 3**), but at a reduced starting point compared to DS.

Discussion

This study was based on the premise that cross-sectional age-related changes in DS neuropathology may resemble or be identical to changes that would occur over time in a single individual with DS. We report here nearly maximal neuronal accumulation of amyloid-β as the first neuropathologic change occurring shortly after birth (identified at 1 year of age) and remaining maximal until the late teens. In this age range there is a concomitant increase in apoptosis [8] and accumulation of APP compared to age-matched controls (noted here). As an increase in severity of apoptosis and APP

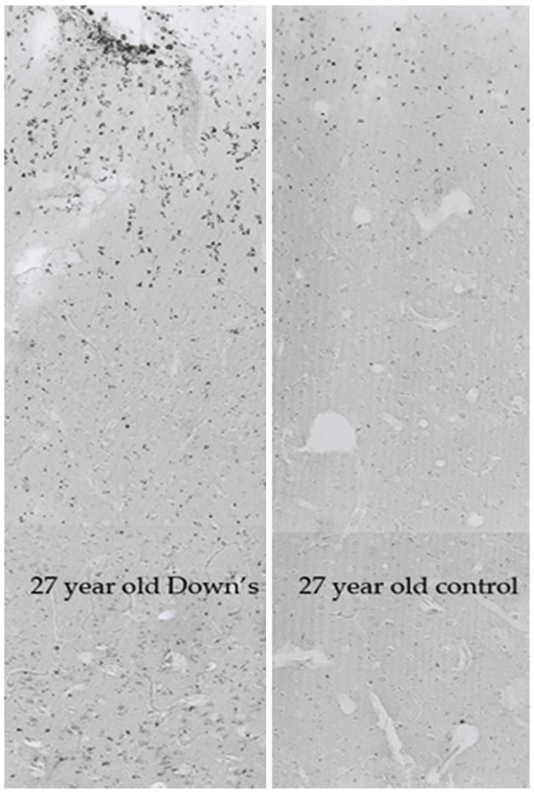

Figure 6. APP immunostaining in young adult DS and normal control individuals. Sections of hippocampal formation were immunostained with 22C11 using standard methods. Montages of the cortical thickness at 10 X magnification is shown for the 27 year old DS subject (left) and the 27 year old normal control (right). The pial surface is at the top of each montage.

accumulation occurs with increasing age, there is a reduction in identifiable neuronal amyloid-bcontemporaneous with initial deposition of amyloid-β in the neuropil as diffuse form SP.

Our intention by assessing 22C11 immunostaining was solely to rule out APP as the epitope identified by 6E10 in DS. We accomplished this but also discerned consistent with findings in cultured astrocytes [11], spherical APP staining was identified in DS and normal control brain tissue immunostained with 22C11. Also consistent with a previous report [12], we identified an age-related increase in APP immunoreactivity in both DS and normal controls.

Amyloid-β is in effect redistributed or redirected from neuronal accumulation to deposition in the neuropil as diffuse-form SP in the early 20's of a DS patient. Simultaneous with SP forma-

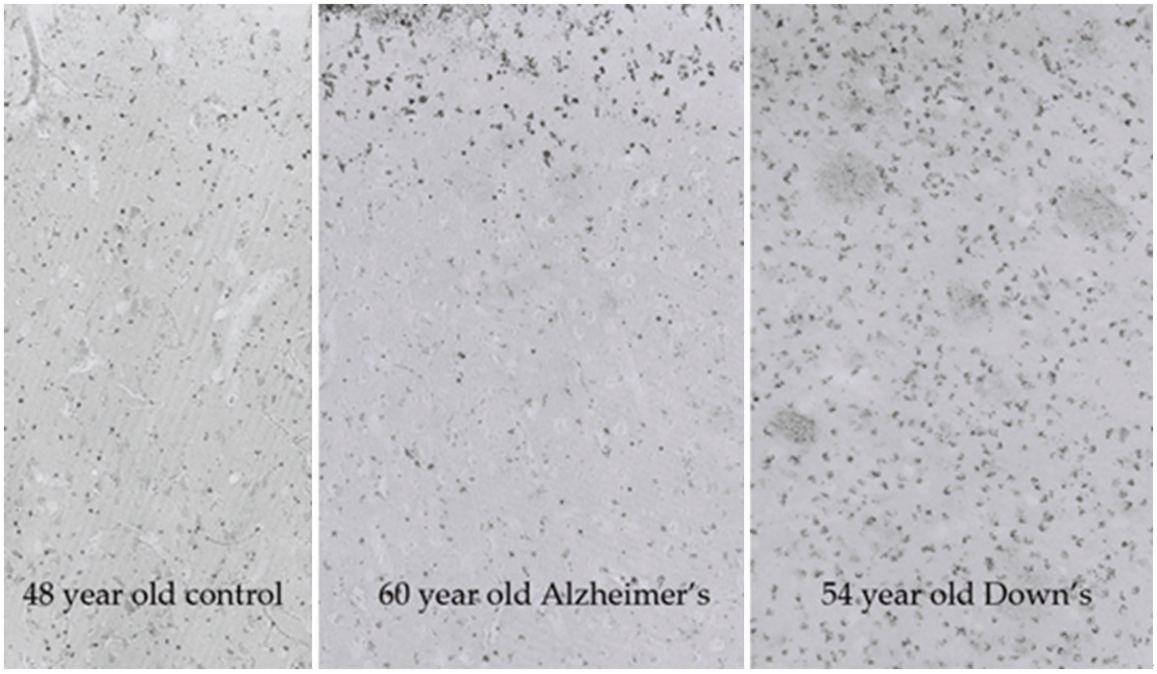

Figure 7. APP immunostaining in AD and older DS and normal control individuals. Sections of hippocampal formation were immunostained with 22C11 using standard methods. Montages of the cortical thickness at 10 X magnification is shown for the 54 year old DS subject (left), the 48 year old normal control (right) and shown for the 60 year old AD subject (center). The pial surface is at the top of each montage.

Table 3. Mean semi-quantitative score for density of APP-immunoreactive spheres in layers II-VI of normal controls and Down's syndrome subjectshippocampal cortex grouped according to age

Age range	Normal control	Down's syndrome
1 - 20 years	0.25 ± 0.42	0.75 + 0.96
21 - 35 years	1.38 ± 0.75	3.17 + 0.29
45 + years	2.88 ± 0.63	5.0

tion there is an identifiable increase in neuronal apoptosis [8] and the onset of microgliosis [9]. In the late 20's and 30's the number of diffuse SP increases dramatically followed by a decrease in number in the late 30's and 40's as they are replaced by increasing numbers of compact, primitive, and dense core SP and subsequently byneuritic SP. This temporal pattern suggests that the diffuse form SP is transformed into these older forms of SP. The evidence also suggests that neuritic SP occur later in the time-line and tend to increase in number as NFT formation begins. We found that both neurites and NFT are immunoreactive for phospho-tau (AT8) and seem to occur more consistently as the prevalence of cognitive impairment increases with increasing age, as established by longitudinal studies among older individuals with DS.

There are two reports of longitudinal assessment of cognitive function in DS that included young individuals. In the early study of 34 individuals between 22 and 56 years of age, no significant change in cognitive function was observed over a three-year period regardless of age at entry to the study [13]. A more recent study investigated 71 DS individuals at age 9 and again at 21 years of age where a significant age-related improvement in cognitive performance was identified and found to be more pronounced the greater the parental/caregiver involvement in providing "problem solving opportunities" [14]. Important to the current study, a cross-sectional investigation ofcognitive performance in 75 DS individuals between 4 and 52 years of age was reported as four age groups; 0-10 years of age (group I), 11-20 years of age (group II), 21-30 years of age (group III), and individuals over 30 years of age (group IV) [15]. In this study the score on essentially every cognitive domain tested was improved in group II compared to group I, was further improved in group III compared to group II, and was reduced in group IV to levels seen in group II [15]. This

suggests that maximal numbers of neurons containing amyloid-bin DS brain are occurring during a period of life associated with improving cognitive performance. This starts two decades prior to the deposition of amyloid-β as diffuse-form SP in DS brain, which in turn occurs nearly 20 years before increased incidence of cognitive impairment.

We posit the concept that neuropathologic alterations occurring first in DS could be closely related to the etiology of the eventual end-stage condition. Based on the evidence developed to date, we regard it as highly unlikely but nevertheless remotely possible that the earliest detectable changes reported herein require 40 years to culminate in sufficient pathologic change as identifiable NFT and neuritic SP, as substrates of progressively severe cognitive dysfunction.

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