Current Gerontology and Geriatrics Research

Aging and Down Syndrome

Guest Editors: Elizabeth Head, Wayne Silverman, David Patterson, and Ira T. Lott

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Editorial Board

Contents

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Editorial

Aging and Down Syndrome

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1. Introduction

The initial clinical description of Down syndrome (DS) was made by Down in 1866 [1] and identified as trisomy of chromosome 21 by Lejeune et al. in 1959 [2]. DS, or trisomy 21, is one of the most common causes of intellectual disability (ID), and recent national prevalence estimates suggest that 14.47 per 10,000 live births are infants with DS, leading to an average of 6037 annual DS births [3]. This represents an increase from previous prevalence rates. Characteristic physical features, deficits in the immune and endocrine systems, and delayed cognitive development [4] can be present in children with DS.

Improvements in medical care for children and adults with DS have led to significant extensions in lifespan and enhanced quality of life. As a consequence, up to 35 years of age, mortality rates are comparable in adults with DS to individuals with ID from other causes. However, after age 35, mortality rates double every 6.4 years in DS, as compared to 9.6 years for people without DS [5], and the currently estimated life expectancy of a 1-year-old child with DS is between 43 and 55 years (depending on the level of disability). Although longevity in adults with DS has been increasing progressively, these increases have been substantially lower for some minority groups [6, 7]. Further, as described by L. Thorpe et al., adults with DS are still disadvantaged compared to adults with other types of ID in terms of mortality rate, with multiple comorbidities being of significant concern (e.g., depression, seizures). Significant contributors to quality of life in aging individuals with DS also include gait disturbances (B. A. Smith et al.) and ophthalmic disorders (e.g., S. J. Krinsky-MC. Hale et al.), both of which increase with age.

2. Dementia and Aging in DS

A key concern to aging adults with DS is the increasing risk for developing Alzheimer's disease (AD). The profile and sequence of cognitive impairments in adults with DS are similar to those seen with AD in the general population [8–13]. Memory processes are affected early in the course of DS dementia [14]. Severe cognitive deterioration, such as acquired apraxia and agnosia, has been reported in 28% of individuals with DS at age 30 years, with a higher prevalence of these impairments in the subsequent years [8, 9]. The earliest manifestations of dementia in DS may involve changes in personality and behavior [15–17]. Pragnosia or socially deficient communication may be an early sign of frontal lobe dysfunction in DS and can represent a striking change from previous well-developed social capacities [18]. Individuals with DS typically show less of a decline in language compared to performance skills associated with aging throughout adulthood as compared to individuals with ID but not DS [19]. The diagnosis of dementia in DS can be challenging against the background of pre-existing intellectual impairment. Standardized criteria for the diagnosis

of dementia in DS include both informant-based and direct measures [16, 20–22]. The severity of preexisting cognitive impairment may also be a predictor of the rate of cognitive deterioration in DS [23].

Potentially modifiable risk factors are now being identified that can lead to enhanced susceptibility to dementia in people with DS [24]. For example, women with DS may develop dementia 10–20 years earlier than women in the general population [9, 25, 26]. One possible mechanism underlying increasing dementia risk for women with DS is suggested by J. H. Lee et al. The study describes a genetic polymorphism in the hydroxysteroid-17β-dehydrogenase gene that is responsible for converting estrone to estradiol. Polymorphisms of this gene may lead to changes in activity of hydroxysteroid-17β-dehydrogenase and modify circulating levels of neuroprotective estrogen. In a cohort of women with DS followed longitudinally, the onset of dementia was linked to three of five single nucleotide polymorphisms (SNPs) in this gene, and women with high-risk SNPs were 2-3 times more likely to develop AD.

3. Neurobiology of Aging in DS

Middle-aged individuals with DS develop AD pathology [25, 27–29], no doubt contributing to their high risk of developing dementia [9, 30]. Still, not every individual with DS will develop dementia. Although clinical signs of dementia are more commonly observed when individuals are over 50 years of age [9, 31–33], by 40 years of age, virtually all individuals with DS have neuropathological changes that are consistent with AD, including senile plaques and neurofibrillary tangles (NFT) [13, 27, 28]. Senile plaques contain the beta-amyloid peptide (Aβ) toxic to neurons and thought to be a causative event in the pathogenesis of AD [34, 35].

DS involves the overexpression of the amyloid precursor protein (APP) on chromosome 21. APP is cleaved sequentially by beta- and gamma-secretases to release Aβ, which forms toxic conformations and aggregates (e.g., senile plaques) in the AD and DS brain [34]. Indeed, the development of AD neuropathology in most individuals with DS after the age of 40 years is considered to be key evidence in support of the amyloid cascade hypothesis as a cause for sporadic AD. Beta-secretase has been characterized as the enzyme beta-amyloid cleaving enzyme (BACE), of which a homologous version, BACE2, is present on chromosome 21 [36]. However, despite increases in BACE2 mRNA in DS brain, protein levels appear similar in DS compared to non-DS brain. Further, BACE2 activity appears to decrease the production of Aβ from APP in contrast to the activity of BACE1. R. L. Webb and M. P. Murphy also describe evidence that BACE activity overall is not increased in the aged DS brain, leading to the conclusion that APP overexpression may be the prime cause of Aβ overproduction. It is also fascinating to consider that despite earlier ages of onset of Aβ deposition in the brain ($\sim$30 years), people with DS are able to compensate for progressive AD neuropathology and many people with DS do not show signs of cognitive decline until their 50s or even later [24]. Similarly, there are people without DS who by imaging studies or autopsy examinations show Aβ deposition in the brain but are clinically normal, suggesting that similar compensatory processes may also occur [37–39].

A novel hypothesis is also provided by A. Reed-Cossairt et al. regarding a role for reduced clearance of Aβ as a consequence of slower cerebrospinal fluid (CSF) turnover. In addition, vascular dysfunction, specifically in the jugular reflux, may be a significant contributor to slowed CSF turnover and the development of dementia in adults with DS. An additional consequence of reduced vascular function may be the development of white matter neuropathology that is typically seen in AD. Many gaps in our knowledge regarding this potential feature of aging in DS that could be modified with appropriate interventions are also discussed by Reed-Cossairt.

Additional neurobiological events that may impact the risk of dementia may also compromise cognition in DS. As summarized by J. P. Lockrow et al., a loss of neurons in the locus coeruleus and basal forebrain (BFCNs) can lead to reductions in two neurotransmitters that play a critical role in learning and memory, norepinephrine, and acetylcholine. Further, these authors provide additional data suggesting that reduced norepinephrine may also lead to increased neuroinflammation and degeneration in the hippocampus (another area critical for memory). Reduced neurotransmitter levels in DS may also lead to a loss of trophic support for neurons in the DS brain with age. Based upon research in mouse models of DS (described in detail by G. N. Vacano et al.), a loss of brain derived neurotrophic factor (BDNF) occurs in response to norepinephrine losses and leads to cognitive deficits. A decrease in BDNF, in turn, has been linked to enhanced vulnerability of neurons to oxidative stress, suggesting a cycle of increasing insults that may eventually lead to neurodegeneration or death.

Oxidative damage has been extensively studied in people with DS, because a significant number (>10) of genes encoding proteins relevant to oxidative damage [40–42] and ROS production located on chromosome 21 [43]. Many of these are overexpressed in DS. SOD1 has been perhaps the most studied protein with regard to ROS metabolism in DS. Increased levels of this endogenous antioxidant without parallel increases in catalase can lead to higher levels of hydrogen peroxide. As reviewed by M. Perluigi et al., oxidative damage may be a significant contributor to neurodegeneration associated with the AD neuropathology seen in DS with advancing age. Specifically, in the aging DS brain, the presence of age-associated Aβ can in turn cause oxidative damage, but there is also evidence that compensatory mechanisms may support normal neuronal function until some threshold is crossed. Further, changes in mitochondrial functioning may produce damaging free radicals that contribute to oxidative stress, given that we see higher levels of mitochondrial DNA mutations in DS (P. E. Coskun and J. Busciglio), although the combination of mitochondrial dysfunction and oxidative stress may lead to adaptive responses in DS, perhaps prior to the development of AD.

Aβ and oxidative damage may contribute to brain inflammation, either independently or in concert. Neuroinflammation, however, has not been studied as extensively as these two other markers of neuropathology and represents an area of focus that may be highly relevant to aging in DS (D. M. Wilcock) [44, 45]. Given that genes involved with neuroinflammation are located on chromosome 21, the neuroinflammatory milieu in DS may be different from AD in the general population. For example, S100β is present in triplicate in DS, is expressed by astrocytes, and is released in response to inflammatory cytokines. As shown in Table 1 of the review by D. M. Wilcock, this is one of multiple genes that could lead to enhanced neuroinflammation in DS, although these genes may also prime the brain towards an M1 inflammatory response. Inflammatory responses may lead to enhanced vulnerability of DS neurons in the presence of Aβ—tangles and oxidative damage—and could be a significant target for intervention. Neuroinflammation has not been fully explored as a function of age in DS and is an active area of research in AD in the general population.

G. Tansley et al. provide novel evidence that circulating levels of 24S-OH-cholesterol, thought to reflect brain levels of cholesterol, are unchanged in aging individuals with DS compared to those without DS. Further, the overall lipid metabolism profile of plasma observed in DS is similar to those without DS. The exception may be brassicasterol levels, which are reduced for older DS individuals compared to those without DS. While this is a very intriguing finding and may reflect AD-associated neuropathology, this is the first report of the effect and further confirmation is required.

4. Pharmacological and Nonpharmacological Intervention Strategies

There are only 5 FDA-approved drugs for the treatment of AD in the general population, and these have met with moderate or little success for the treatment of AD in DS [46–51]. This suggests that other disease-modifying approaches are going to be critically important for future therapeutics. Prevention, however, may be the most promising approach to healthy aging in DS and may include both pharmacological and nonpharmacological interventions.

Critically important to the development of novel therapeutics or prevention strategies is the use of mouse models for DS where promising strategies can be first tested. There are several mouse models for DS that capture developmental and aging-associated phenotypes (G. N. Vacano et al.). Although DS is a complex genetic disorder, careful dissection of the role of individual or groups of genes to the DS phenotype provides an exciting approach for the development of new interventions. In combination with existing mouse models for AD, promising new pharmacological or nonpharmacological treatments may be identified. However, it is also critical to note that translation of outcomes from mouse studies to human clinical trials is not necessarily direct, but the mouse studies provide important proof of principle outcomes that can be pursued.

As one example of using this approach, J. P. Lockrow et al. review studies suggesting that the norepinephrine precursor L-threo-DOPS (Droxidopa) can improve learning and memory in DS mice and may be a possible target for DS clinical trials addressing improvement of age- and AD-associated cognitive dysfunction. As a parallel component to enhancing norepinephrine function, neuroinflammation appears to be intimately linked to the levels of this neurotransmitter and increased in DS aging mice.

Another pharmacological approach to preventing AD in people with DS may be to modify the production of Aβ due to overexpression of APP (R. L. Webb and M. P. Murphy). Clinical trials are currently addressing the reduction of BACE activity in sporadic cases of AD (http://www.clinicaltrials.gov/), but gamma-secretase inhibitors may not be a viable option given adverse side effects. An intriguing report in a mouse model of DS showed that reducing BACE activity in young animals reduced learning and memory deficits, suggesting that APP overexpression and production of Aβ may not only be involved with AD development with age but also contribute to intellectual disability in younger individuals [52].

Reducing oxidative damage (which is a lifelong issue for DS) may require a multitargeted approach that is preventative in nature, given that supplementing demented adults with DS with antioxidants (or individuals with AD in the absence of DS) has shown little or no benefit to clinical outcomes [53]. Indeed, compensatory mechanisms at younger ages in DS may be enhanced by antioxidant supplementation (M. Perluigi and D. A. Butterfield). Focusing on mitochondrial dysfunction is also a promising approach, as these organelles are the primary producers of reactive oxygen species (P. E. Coskun and J. Busciglio). In addition, isolated mitochondria have a higher rate of DNA mutations, suggesting a progressive exacerbation in mitochondrial function in DS. Given this evidence for mitochondrial dysfunction in DS, there are several possible interventions that may reduce age-associated declines (e.g., dietary changes and/or supplementation with mitochondrial cofactors). A combinatorial approach may be particularly valuable for adults with DS who may benefit from a supplement including both antioxidants (e.g. vitamins E and C) and mitochondrial cofactors (e.g., lipoic acid, acetylcarnitine). However, it may be critical to use these approaches as a preventative measure rather than as a treatment protocol for AD in DS [53]. Given the interaction between Aβ, oxidative damage and neuroinflammation, and the relative paucity of data regarding inflammation in the aging DS brain, unexplored potential new targets for intervention may exist (D. M. Wilcock).

Several key issues highlighted in this special issue topics suggest that lifestyle modification and regular health monitoring may also lead to successful aging in people with DS. For example, although older adults show decreased stability and efficiency in gait during walking, evidence for adaptation suggests potential for improvements with appropriate interventions (B. A. Smith et al.). Changes in gait should be taken into consideration, as they may lead to less physical activity and/or functional decline.

A substantial number of people with DS develop ophthalmic disorders, affecting up to 50% of adults between 50 and 59 years of age (S. J. Krinsky-MC. Hale et al.).

The development of age-associated visual deficits occurs at younger ages in DS than that in the general population. The presence of ophthalmic disorders is higher in DS individuals with more severe intellectual disability, leading to additional challenges and significant functional consequences. Interestingly, in S. J. Krinsky-MC. Hale's report of longitudinally followed individuals with DS, cataracts were the most frequent problem in older adults but were not associated with the level of ID. However, the presence of cataracts does compromise functioning and readily available treatments can improve quality of life for affected individuals with DS.

Diet may be a very important area to consider for healthy aging in DS. For example, the reduced levels of brassicasterol reported by G. Tansley et al. may be modifiable by diet. Whether this would mechanistically reduce the development of AD or impact on longitudinal changes in cognition has yet to be determined. Additionally, managing estrogen levels in aging women with DS may help to reduce their risk for developing AD dementia, but an individualized approach including genetic risk as determined by the presence of SNPs on the HSD17B1 gene may need to be incorporated (J. H. Lee et al.).

5. Summary

This special issue of the *Current Gerontology and Geriatrics Research* journal was intended to cover selected issues defining the concerns faced by adults with Down syndrome as they grow older. As can be seen from this selection of papers, substantial gaps in our knowledge of the aging process in people with DS continue to persist and are critically important to address. Further, approaches for maintaining healthy aging in individuals with DS may also inform strategies for enhancing quality of life for other adults with ID or indeed in the general population. Ongoing longitudinal studies that monitor changes in health status, cognition, function, the development of dementia, and mortality will all be critically important for informing the development of policies and interventions to promote healthy aging in DS. A key challenge to aging adults with DS is the increasing risk for developing dementia; yet our social and medical infrastructure is not as well prepared for providing care to adults with DS as they age relative to the outstanding support available to children with DS and their families. The use of FDA-approved treatments for AD in the general population has met with limited success in people with DS [46]. Therefore, it is critically important to explore novel interventions and potential prophylactic approaches that may provide individuals with DS the best possible opportunity to age gracefully. Such interventions along with further study of aging, dementia, and Alzheimer disease in DS are likely to be of fundamental importance in understanding AD in the general population.

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Research Article

Gait Parameter Adjustments for Walking on a Treadmill at Preferred, Slower, and Faster Speeds in Older Adults with Down Syndrome

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The combined effects of ligamentous laxity, hypotonia, and decrements associated with aging lead to stability-enhancing foot placement adaptations during routine overground walking at a younger age in adults with Down syndrome (DS) compared to their peers with typical development (TD). Our purpose here was to examine real-time adaptations in older adults with DS by testing their responses to walking on a treadmill at their preferred speed and at speeds slower and faster than preferred. We found that older adults with DS were able to adapt their gait to slower and faster than preferred treadmill speeds; however, they maintained their stability-enhancing foot placements at all speeds compared to their peers with TD. All adults adapted their gait patterns similarly in response to faster and slower than preferred treadmill-walking speeds. They increased stride frequency and stride length, maintained step width, and decreased percent stance as treadmill speed increased. Older adults with DS, however, adjusted their stride frequencies significantly less than their peers with TD. Our results show that older adults with DS have the capacity to adapt their gait parameters in response to different walking speeds while also supporting the need for intervention to increase gait stability.

1. Introduction

Persons with Down syndrome (DS) have lower tone and higher ligamentous laxity than their peers with typical development (TD), requiring them to find somewhat different solutions to control gait over their lifespan. For preadolescents with DS, merely increasing step width as compared to their peers with TD seems adequate to provide stability for walking overground at their self-selected speed [1, 2]. However, in response to the effects of aging, and at an earlier age than observed in the population with TD, adults with DS make additional changes to maintain gait stability while walking overground at their self-selected speed. Adults with DS aged 35–62 years walked slower, with shorter, wider strides and increased stance and double support periods than their age-matched peers with TD [3]. There are a number of factors known to affect gait patterns in older adults with TD that may contribute to the observed gait patterns in adults with DS, including neurophysiological changes associated with aging [4, 5], sedentary lifestyle [6], osteoarthritis [7], obesity [8], and Alzheimer's type dementia [9–14].

While preadolescents with DS only need to make minimal adaptations (adjusting only step width) to their gait pattern to achieve stability while walking overground at their self-selected speed, they make many more adjustments when asked to walk on a treadmill [1, 2]. We found that preadolescents with DS shortened, widened, and increased stride frequency more than their peers with TD, at their preferred speed and speeds slower and faster than preferred [1, 2]. We attribute the increased adjustment of gait parameters for persons with DS during treadmill walking to the novelty and greater stability challenge presented by the treadmill [1, 2]. Our purpose here was to examine how older adults with DS adapt their gait when asked to

Table 1: Anthropometric measurements for adults with Down syndrome (DS) and typical development (TD), mean (standard deviation).

Group	Height (m)	Weight (kg)	Age (years)
DS adults	1.51 (0.08)	74.9 (22.9)	43.9 (8.7)
TD adults	1.59 (0.05)	64.5 (15.0)	43.6 (6.9)

walk on a treadmill at their preferred speed and at speeds slower and faster than preferred. The ability to adapt gait in response to changing environmental contexts is important in daily function. Further, it is possible that screening for adaptability, in a task like treadmill walking, might provide an early window on the gradual deterioration of gait function in everyday life activities. In our experimental design the treadmill increased the stability challenge beyond that of typical gait function, while slower and faster than preferred walking speeds pushed the participants' systems further out of their comfort zone and required gait adaptation.

2. Method

2.1. Participants. Twenty adults between 35 and 62 years of age participated in the study, 10 with DS and 10 with TD. Participants were matched for age (see Table 1). Adults with TD were healthy, lived independently, and were recruited through a university research volunteer website. Participants with DS were recruited from community support groups or through local supervised residences.

Of the 10 adults with DS, 6 lived in supervised residences while 4 lived with their parents. In terms of assistance needed for activities of daily living, 5 were able to independently bathe, dress, and feed themselves while 3 needed minimal and 2 needed moderate assistance with these activities. Five were able to shop independently while 2 required minimal assistance and 3 needed total assistance. Five of the adults with DS reported regular physical activity, mostly walking, 2–7 days per week, 10 minutes to 1 hour in duration. Eight had jobs consisting of light physical activity (e.g., housekeeping, packing boxes) from 3 to 30 hours per week.

From their self and caregiver-reported health histories, the following health conditions were reported for the adults with DS (number reporting condition in parentheses): corrected vision deficits (8), obesity (6), heart murmur and/or valve repair surgery (5), occasional pain in hips and legs (6), corrected hearing deficits (5), hypothyroidism (5), hyperlipidemia (3), pes planus (4), dementia (2), high blood pressure (1), renal transplant (1), syncope (1), and seizures (1). For their overall health status, self-ratings were as follows: excellent (2), very good (4), good (1), okay (1), and declined to answer (2).

2.2. Procedures. All procedures were approved by the University of Michigan Institutional Review Board. When participants and their parents or caregivers came to the laboratory we explained all procedures and asked them to sign an assent or consent form, as appropriate. Next, participants changed into fitted shorts, a tank top, and removed shoes and socks. We attached markers (2.5 cm diameter) bilaterally to the skin at temporomandibular joint, acromion process, lateral humeral epicondyle, styloid process, greater trochanter, femoral condyle, 10 cm above the lateral malleolus, heel bony prominence, and third metatarsophalangeal joint. We placed EMG electrodes over the muscle bellies of the tibialis anterior, gastrocnemius, rectus femoris, and biceps femoris of the left leg. For the questions addressed in this paper EMG results will not be discussed.

We used a 6-camera Vicon Peak Motus real-time system (Vicon Motion Systems Centennial, CO) to collect reflective marker position data at 60 Hz as participants walked overground and on the treadmill. After 4–6 practice trials, participants walked at their preferred speed over a 5.3 meter GAITRite (CIR Systems, Inc., Havertown, PA) mat to the other side of the room. Each participant repeated this condition 4–6 times until we obtained 4 passes with all markers visible.

After overground data collection, the treadmill was moved into the calibrated space. From the overground trials we used GAITRite software to calculate average speed, which we used to set the treadmill speed for each individual performer. Participants were spotted while walking on a treadmill (Parker Brand) for 30-second data collection periods at 40%, 75%, and 110% of their comfortable overground walking speed. We defined the 75% speed on the treadmill as preferred pace [1, 2] and the 40% and 110% speeds as slower and faster than preferred. Participants practiced at each speed until they were able to walk comfortably (by their report) and maintain upright posture without holding onto the treadmill handrail. One participant with DS was afraid to walk at the fastest speed and so only completed the 40% and 75% treadmill speeds.

Anthropometric measurements were collected for calculation of dimensionless values. We measured weight (Healthometer beam scale), standing height (GPM anthropometer), sitting height, and length of the upper arm, arm, thigh, shank, and foot.

Participants with DS came to our laboratory for approximately 2 hours. We scheduled two sessions for adults with DS in order to keep each visit shorter and less stressful. During the first visit they walked overground followed by practice walking on the treadmill at their 75% speed. During the second visit we measured body segments and participants walked on the treadmill at 40%, 75%, and 110% speeds. Because adults with TD learn tasks faster and were more at ease with the testing conditions, they performed all tasks in one visit.

2.3. Data Analysis. We converted raw kinematic data to 3D data using PeakMotus software and a 6 Hz second-order Butterworth filter. We used a custom-written MATLab program (Mathworks Natick, MA) to identify initial foot contact and toe off events based on vertical acceleration of heel markers and horizontal accelerations of toe markers [15]. We used identified gait events, 3-D data, and anthropometric measurements to calculate dimensionless stride frequency, stride length, and step width values. Dimensionless values were used to account for leg length and leg length/trunk

ratio differences [3]. For formulas and definitions of dimensionless parameters we used please see the appendix. Percent stance was calculated as the percent of each stride cycle between heel contact and toe off and thus included times when all or part of the foot was in contact with the ground.

We used SPSS (SPSS Inc., 233 S. Wacker Dr., Chicago, IL) version 19.0 for statistical testing. We set our alpha level of significance at 0.05. We used a multivariate analysis of variance (MANOVA) with repeated measures on speed and Bonferroni adjustments for multiple comparisons to test for a group effect, speed effect, and a group-by-speed interaction. Dependent variables were average dimensionless stride frequency, dimensionless stride length, dimensionless step width, and percent stance values for each participant at each speed.

3. Results

Table 2 presents absolute stride frequency, stride length, step width, and percent stance values by group and speed. The absolute values are provided to allow comparison to extant literature; however, we made our group comparisons based on dimensionless values to account for anthropometric differences between the populations.

Overall, the MANOVA demonstrated a significant group-by-speed interaction (Wilks' Lambda = 0.20, $F[8, 10] = 5.06$, $P = 0.01$). There were also significant main effects of group (Wilks' Lambda = 0.20, $F[4, 14] = 13.85$, $P > 0.01$) and speed (Wilks' Lambda= 0.01, $F[8, 10] = 88.65$, $P > 0.01$), which are not interpreted due to the significant group-by-speed interaction.

Follow-up univariate analysis showed that the significant group-by-speed interaction was due to differences in adjustment of dimensionless stride frequency (Huynh-Feldt $F(1.34, 22.8) = 13.86$, $P > 0.01$). Stride frequency increased from 40% to 75% to 110% speeds in both groups (pairwise comparisons $P < 0.01$ for all); however, there was less adjustment of stride frequencies at the slower speed by participants with DS as there was a significant group difference in dimensionless stride frequency at the 40% speed ($F[1, 17] = 8.74$, $P = 0.01$) but not at the 75% ($F[1, 17] = 3.34$, $P = 0.09$) or 110% ($F[1, 17] = 0.84$, $P = 0.37$) speeds. Figure 1 shows higher dimensionless stride frequency in the DS group compared to the TD group at the 40% speed but not at the 75% or 110% speeds.

Adjustments of stride length, step width, and percent stance were consistent between the DS and TD groups. Figure 2 demonstrates that dimensionless stride lengths increased from the 40% to 75% to 110% speeds in both groups (pairwise comparisons $P < 0.01$ for all) and were shorter in the DS group at all speeds (40% = $F[1, 17] = 44.35$, $P > 0.01$; 75% ($F[1, 17] = 21.93$, $P < 0.01$; 110% ($F[1, 17] = 18.68$, $P < 0.01$). As shown in Figure 3, dimensionless step widths, did not change by speed in either group (pairwise comparisons $P > 0.05$ for all) but were greater in the DS group at all speeds (40% = $F[1, 17] = 13.03$, $P = 0.01$; 75% ($F[1, 17] = 12.94$, $P = 0.01$; 110% ($F[1, 17] = 17.29$, $P = 0.01$). Percent stance (Figure 4) decreased from the 40% to 75% to 110% speeds in both groups (pairwise

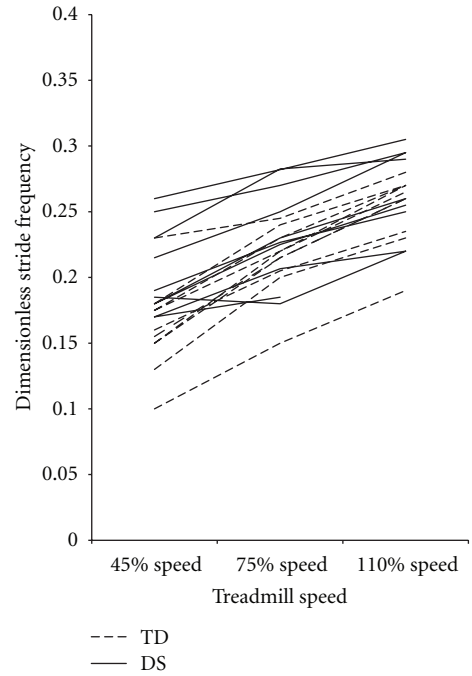

Figure 1: Mean dimensionless stride frequency values for each participant, by group and speed. Dimensionless stride frequency values increased as speed increased and were significantly different between groups at the 40% speed but not at the 75% speed or the 110% speed. DS: Down syndrome, TD: typical development.

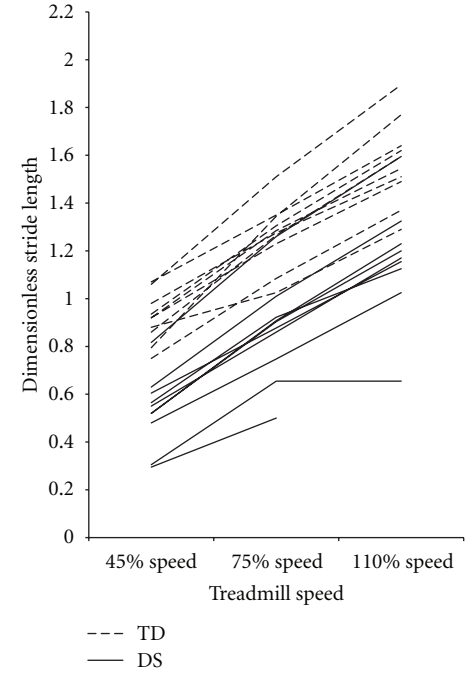

Figure 2: Mean dimensionless stride length values for each participant, by group and speed. Dimensionless stride length values increased with speed and were significantly different between groups at all speeds. DS: Down syndrome, TD: typical development.

Table 2: Absolute treadmill gait variables for adults with Down syndrome (DS) and typical development (TD), mean (standard deviation).

Group	Speed	Treadmill speed (m/s)	Step width (m)	Stride length (m)	Stride frequency (strides/s)	Percent stance (%)
DS adults	40%	0.28 (0.09)	0.14 (0.06)	0.37 (0.13)	0.77 (0.12)	80.6 (3.7)
	75%	0.54 (0.15)	0.15 (0.06)	0.60 (0.16)	0.91 (0.16)	75.6 (4.1)
	110%	0.80 (0.20)	0.15 (0.06)	0.79 (0.19)	1.01 (0.12)	71.9 (3.8)
TD adults	40%	0.39 (0.07)	0.10 (0.03)	0.68 (0.09)	0.59 (0.13)	77.5 (3.6)
	75%	0.73 (0.12)	0.09 (0.03)	0.94 (0.10)	0.78 (0.10)	73.2 (2.2)
	110%	1.07 (0.18)	0.09 (0.02)	1.17 (0.12)	0.91 (0.11)	69.4 (1.9)

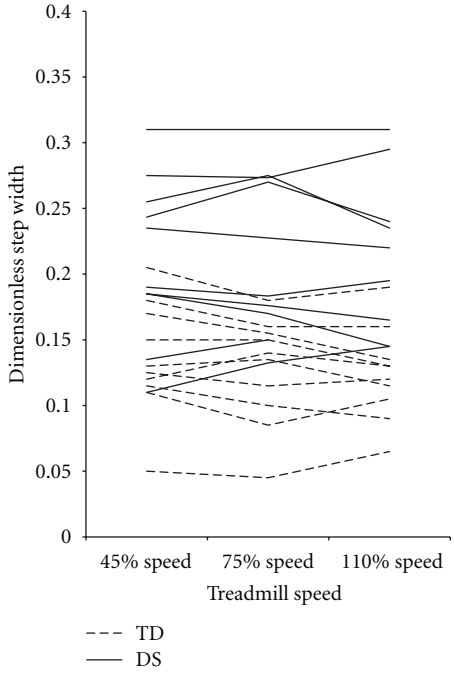

Figure 3: Mean dimensionless step width values for each participant, by group and speed. Dimensionless step width values did not change with speed and were significantly different between groups at all speeds. DS: Down syndrome, TD: typical development.

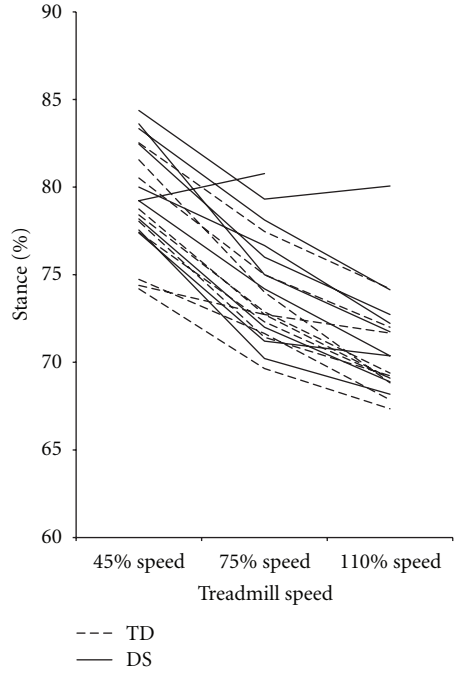

Figure 4: Mean percent stance values for each participant, by group and speed. Percent stance values decreased as speed increased and were not different between groups at any speed. DS: Down syndrome, TD: typical development.

comparisons $P < 0.01$ for all) and was not different between groups at any speed (40% = $F[1, 17] = 0.30$, $P = 0.59$; 75% ($F[1, 17] = 2.65$, $P = 0.12$; 110% ($F[1, 17] = 1.40$, $P = 0.25$).

4. Discussion

Overall, we found that older adults with DS in this sample were able to adapt their gait to slower, and faster than preferred treadmill speeds, by maintaining their stability-enhancing foot placements at all speeds. Previous work has shown that older adults with DS demonstrate slower preferred-speed overground gait with stability-enhancing adaptations of shorter strides, wider step widths, and increased stance and double support phases compared to adults with TD [3]. Here we found consistent stability-enhancing differences (shorter stride lengths and wider step widths) during treadmill walking for adults with DS at preferred, slower, and faster treadmill-walking speeds.

Both older adults with TD and DS adapted their gait patterns similarly in response to faster and slower than preferred treadmill-walking speeds. To adapt gait to the faster treadmill speed, all participants increased stride frequency and stride length, maintained step width, and decreased percent stance. To adapt gait to the slower treadmill speed, all participants decreased stride frequency and stride length, maintained step width, and increased percent stance. Older adults with DS, however, adjusted their stride frequencies significantly less than their peers with TD. In particular, adults with DS showed less decrease in stride frequency for the slower treadmill speed. Less ability to adjust stride frequency indicates some difficulty in adapting movement speed in adults with DS. Our results show they were more able to adjust foot placements (step width and stride length) than they were to adjust the movement speed of their stride (as reflected by stride frequency). This finding is consistent with previous reports of difficulty adjusting movement speed

in persons with DS; however, it is also important to note that movement speed can be adapted following task practice [16–18].

Our findings are mostly consistent with previous research on treadmill walking in younger adults with DS (ages 19–44 years). Our results agree with those of Agiovlasitis and colleagues [19], who found that both groups (DS and TD) attained faster speeds by increasing step length and decreasing step time. Our results both support the use of stability-enhancing foot placements, although the precise findings differ slightly. In their study, adults with DS walked with faster, shorter-duration steps at all speeds and shorter steps at slow speeds, each of which would enhance stability by increasing the overall proportion of time spent with the foot in contact with the ground. Our studies do find conflicting results, however, for step width. In their study, adults with DS did not take wider steps than adults with TD (absolute or normalized to leg length). This may be due to their younger participants who appear to be more regularly physically active than ours or to slightly different procedures for normalization [19]. We consistently find wider step width for participants with DS compared to their peers with TD across the lifespan from new walkers to preadolescents to older adults, both for absolute values and values normalized to leg length [1–3, 20, 21]. We have not, however, tested participants between 10 and 35 years of age. Rigoldi and colleagues [22] reported wider step width in children (M 9.2 yrs, SD 2.5 yrs), teenagers (M 16.7 yrs, SD 3.2 yrs), and adults (M 37.5 yrs, SD 2.5 yrs) with DS during self-paced overground walking.

The gait adaptations we observed here in older adults with DS are very similar to what we found for preadolescents. In our previous study with the same treadmill walking measurements, preadolescents (ages 8–10 years) with DS and TD increased stride length and decreased step width as treadmill speed increased, although step width, even when normalized to leg length, remained wider in participants with DS. Preadolescents with DS demonstrated higher stride frequency than the group with TD and all participants decreased their stride frequency more at slower speeds [2]. Additionally, we used an escapement-driven inverted pendulum and spring model to measure global dimensionless stiffness and impulse values as a reflection of efficiency and stability of gait. We found higher levels of stiffness and impulse during treadmill walking for preadolescents with DS compared to their peers with TD, reflecting an increased need for stability and a less efficient gait pattern overall [1].

Although we did not measure stability and efficiency of gait directly in this study, our results do support the idea that older adults with DS demonstrate decreased stability and efficiency of gait. Adults with DS walk slower with shorter, wider strides than their peers with TD, consequently covering less ground with each stride likely using more energy to produce a gait pattern over a given distance. This pattern is robust, observed here consistently across different treadmill-walking speeds. Our results complement those of researchers who directly measured higher metabolic energy expenditure and lower cardiorespiratory function during treadmill walking in adults with DS [23, 24].

In previous work, we used nonlinear measures to analyze patterns of gait variability across the lifespan in persons with DS and found that older adults with DS demonstrated decreased adaptability of gait compared to their preadolescent peers with DS [25]. Our results here show that older adults with DS, despite less adaptability than their younger peers, still retain some capacity to adapt their gait parameters in response to different treadmill speeds. The older adults with DS in our study showed the ability to adapt their gait in response to changing environmental contexts, an important ability for daily function, and likely a feature that could be improved upon with appropriate intervention.

In addition to adaptability of gait, our results also support the need for intervention to increase gait stability as older adults with DS continue to demonstrate here, as well as previously [3], a need for increased walking stability earlier in life than adults with TD. This need for increased gait stability likely emerges from many factors. Age-related physiological changes may contribute [4, 5], as well as a sedentary lifestyle [6], osteoarthritis [7], and obesity [8]. Additionally, adults with DS experience a loss of oligodendrocytes in the basal ganglia [26], an area of the brain known to contribute to movement control. Abnormally high levels of oxidative stress [27] may induce a vulnerability for the very high levels of Alzheimer's type dementia (neurofibrillary tangles and plaques) observed in adults with DS over 40 years of age [9–11]. These physiologic and neural changes are experienced in addition to the inherent lifelong stability challenges of ligamentous laxity and hypotonia, with the culmination of these many effects likely contributing to less stable gait patterns in adults with DS.

Although researchers have acknowledged that there are multiple contributing factors to falls in adults with DS [28, 29], a causal relationship between decreased gait stability and adaptability and an increased risk of falls in adults with DS has not yet been shown. In fact, even the presence of increased fall risk in adults with DS has not been well documented. One group of researchers reported that adults with DS were less likely to fall than adults with epilepsy or autism [29]; however, their rate of falls was not compared to adults with TD. We previously reported that out of 14 adults with DS between the ages of 35 and 65 years who demonstrated high variability and decreased stability of gait, 6 reported a history of falls and 8 did not [21]. Prospective studies of fall risk in adults with DS, however, are lacking.

In addition to prospectively studying the risk of falls in adults with DS, future work is also necessary to determine how different factors relate to observed gait changes and mechanisms of falls in adults with DS. Our results show a continuum of gait performance in adults with DS, this is particularly apparent in the overlapping group values for dimensionless stride frequency (Figure 1) and dimensionless step width (Figure 3). Some adults with DS performed similarly to adults with TD while others did not. We did not run analyses on the relation between participants' specific characteristics and the dependent variables because our sample was too small to allow meaningful correlations. In the future, however, it is important to determine the weighted contribution of health status and lifestyle factors such as

dementia, obesity, and inactivity to efficiency of gait patterns, falls, and quality of life. Once the relationship among factors is defined, appropriate (and likely multifactorial) interventions can be designed and tested in an effort to positively affect the health, mobility status, and quality of life of adults with DS. Although they likely will not be able to adapt to the same degree as preadolescents with DS as the challenges they face are greater, our results here support that adults with DS could likely improve and maximize the efficiency, stability, and adaptability of their gait with appropriate intervention.

Appendix

Formulas for normalization to dimensionless values are

$$\hat{\mathbf{w}}_{\text{STEP}} = \frac{\mathbf{w}_{\text{STEP}}}{\mathbf{l}_O}, \tag{A.1}$$

$$\hat{\mathbf{l}}_{\text{STRIDE}} = \frac{\mathbf{l}_{\text{STRIDE}}}{\mathbf{l}_O}, \tag{A.2}$$

$$\hat{\mathbf{f}}_{\text{STRIDE}} = \frac{\mathbf{f}_{\text{STRIDE}}}{\sqrt{\mathbf{g}/\mathbf{l}_O}}, \tag{A.3}$$

where $\hat{\mathbf{w}}_{\text{STEP}}$ (step width), $\hat{\mathbf{l}}_{\text{STRIDE}}$ (stride length), and $\hat{\mathbf{f}}_{\text{STRIDE}}$ (stride frequency) are the converted gait variables, $\mathbf{l}_O$ is leg length (sum of thigh length and shank length), and $\mathbf{g}$ is acceleration due to gravity.

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Clinical Study

Clinical Predictors of Mortality in Adults with Intellectual Disabilities with and without Down Syndrome

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Background. Mood, baseline functioning, and cognitive abilities as well as psychotropic medications may contribute to mortality in adults with and without Down Syndrome (DS). *Methods.* Population-based (nonclinical), community-dwelling adults with intellectual disabilities (IDs) were recruited between 1995 and 2000, assessed individually for 1–4 times, and then followed by yearly phone calls. *Results.* 360 participants (116 with DS and 244 without DS) were followed for an average of 12.9 years (range 0–16.1 years as of July 2011). 108 people died during the course of the followup, 65 males (31.9% of all male participants) and 43 females (27.6% of all female participants). Cox proportional hazards modeling showed that baseline practical skills, seizures, anticonvulsant use, depressive symptoms, and cognitive decline over the first six years all significantly contributed to mortality, as did a diagnosis of DS, male gender, and higher age at study entry. Analysis stratified by DS showed interesting differences in mortality predictors. *Conclusion.* Although adults with DS have had considerable improvements in life expectancy over time, they are still disadvantaged compared to adults with ID without DS. Recognition of potentially modifiable factors such as depression may decrease this risk.

1. Introduction

Although much improvement has occurred during the last century, mortality rates in people with childhood onset intellectual disabilities (IDs) are still higher than those of the general population, especially in younger adults in their 20s and people with Down syndrome (DS) [1]. In general populations, increased age is known to be an important predictor of increased mortality, as is male gender, although some data suggests that males with DS may have a relative survival advantage [2]. In general, mortality rates are lower in community samples, although this may not be true for those with severe disabilities, whose needs may be met less well in the community [3, 4].

Also of potential significance to mortality is the Intelligence Quotient (IQ). Among people with ID, those with the most severe impairment were found in Patja et al's cohort study (previously referenced) to have significantly lower life expectancy, whereas those with mild ID had similar life expectancy to the general population. This difference in life expectancy is likely related to increased severity of underlying medical illness in those with the greatest intellectual impairment.

In the general population, excess mortality (especially due to cardiac and respiratory diseases) has been found in those with major mental illness [5], case level depression [6] and those who have depressive symptoms and medical illness such as unstable angina [7]. Depressive symptoms have been linked to decreased total active (and total) life expectancy [8, 9], with some suggestions by Win et al. that some of this is mediated by physical inactivity. Other reports have linked autonomic dysfunction and inflammation to the increased cardiovascular mortality risk associated with depression [10]. Depressive symptoms have also been linked

to an increased risk of dementia in the general population [11] and in a DS population [12], but reasons for this association have not fully been clarified. It is possible that depression is itself a very early manifestation of the development of a degenerative process such as Alzheimer's disease, but it may also exert (directly or indirectly) adverse effects on the biological structures in the brain, causing or accelerating the degenerative process of dementia (although recent neuropathological work by Tsopelas et al. [13] makes the latter explanation less likely). Dementia in turn has been associated with increased mortality in the general population [14].

Other potential contributors to mortality include the use of psychotropic medications. There has been particular concern about the use of antipsychotic medications in people with dementia, with some studies (but not all) suggesting increased mortality and strokes (see review by [15]). Associations between antipsychotics and adverse health outcomes are clearly not specific to people with dementia, as shown in general population studies of increased sudden cardiac death related to antipsychotics [16]. Adults with ID are commonly prescribed antipsychotics [17–20], often for behavioural problems, and may therefore be particularly impacted by this adverse outcome.

Anticonvulsants are another potential contributor to increased mortality. Although epilepsy itself is associated with increased mortality including sudden unexplained death [21–23] and new onset seizures are thought to be markers for cognitive decline in people with DS (see review in [24]), recent epidemiologic and placebo-controlled trials data suggest that increased rates of death, especially violent death including suicides, may be related to anticonvulsants themselves [25, 26]. As anticonvulsants are used frequently in people with ID, who have a high rate of epilepsy as well as behavioral problems for which anticonvulsants are used, this might be an important and potentially modifiable factor in improving mortality rates.

The population-based (nonclinical), Intellectual Disability and Aging Study was designed in the early 1990s to fill gaps in clinical understanding of longitudinal cognitive and functional changes as well as mortality patterns in adults with Down Syndrome (DS). After the methodology was discussed at various university and community forums, adults with ID but not DS were added to the study to provide an appropriate control group. This paper focuses particularly on baseline contributors to mortality.

2. Methods

Appropriate authorization for whole study was obtained from the University of Saskatchewan Ethics Committee. Procedures followed were in accordance with the ethical standards of the Helsinki Declaration of 1975, as revised in 2000 [27]. Letters requesting participation of any adult with caregiver (or family) defined childhood onset intellectual disability aged 18 years and over were sent in 1995 to all provincial community services (group homes, independent living organizations, supportive work settings) designated for adults with ID. There were no additional exclusion criteria. Information was provided about the study, and clinical coordinating staff was asked to forward enclosed consent forms to potential participants or their usual substitute decisionmakers for medical decisions. Participants who clearly understood the process of the study were asked to provide their own consent. If the potential participant assented but obviously lacked capacity for full, informed consent, the person who normally consented to health care interventions provided consent. If there was assent but partial or unclear capacity to consent, both the person and their usual medical decisionmaker would provide consent. No participants were included whose family or immediate caregivers voiced opposition to participation after full information was provided. Participants or their substitute decisionmakers mailed signed consent forms back in provided envelopes. Research staff made periodic phone contact as necessary with community service providers to provide further information about the study and to answer questions about eligibility and appropriate provision of consent. One exception was made to accept a 17 year old participant whose substitute decision-maker mailed in a consent form.

Once completed consent forms were received, questionnaires addressing basic demographics, residential information (type of living situation), name of family physician, psychiatric care, name of social services case manager, basic health information (including suspected or confirmed dementia), seizure history and frequency of current seizures, medication use, estimated level of disability (profound, severe, moderate, mild, and borderline), and most recent IQ score before the age of 18 were mailed to the care provider who was most familiar with the participant. Care providers were also mailed copies of standardized caregiver-rated instruments (described in the following) to complete. After receiving the completed questionnaires and instruments, research staff contacted care providers to assess the participant's ability and/or willingness to engage in direct interviews and testing. 276 participants eventually had at least one direct assessment consisting of a variety of instruments, which are not described here as they were not used in the analysis forming the basis of this paper. All information was reviewed by the primary investigator, and additional contacts were made with care staff, families, and medical staff as necessary to confirm accuracy of information.

To establish to representativeness of our initial sample, we obtained baseline 1995 service provision data (numbers and age distribution) from the division of the Department of Social Services responsible for people with ID, as our sample was drawn from people participating in those services.

No financial reimbursement was given to participants, but at each wave of direct data collection a printed certificate of participation was presented.

2.1. Data Collection. Formal instruments used in the study were chosen for their ease of administration, acceptability, validity, and psychometric data and had to be further amended by the funding and manpower available. Final instruments included in the caregiver package were Evenhuis'

Dementia Questionnaire for Persons with Mental Retardation (DMR: [28]) and the Reiss Screen for Maladaptive Behavior [29].

2.2. Dementia Questionnaire for Persons with Mental Retardation (DMR). The Evenhuis questionnaire was chosen for the evaluation of cognitive and functional decline in those with ID as it was one of the practical and well-known caregiver-rated instruments designed for this purpose, and our study did not have resources to provide comprehensive individualized dementia diagnoses to participants across the province. The standardized 50-item instrument is based on the dementia criteria in the DSMIII-R [30] but was adapted to allow for easier scoring in those with baseline intellectual disabilities. Higher scores on the DMR (based on behaviour over the last three months) indicate more impairment. Subscales of the DMR include short-term memory, long-term memory, spatial and temporal orientation, speech, practical skills, mood, activity and interest and behavioural disturbance.

The DMR subscales themselves have been summed to derive two major subscales: the Sum of Cognitive Scores (SCS: short-term memory, long-term memory, spatial and temporal orientation), which have a score range of 0 to 44, and the Sum of Social Scores (SOS: speech, practical skills, mood, activity and interest and behavioral disturbance), which has a range of 0 to 60. The preferred use of the DMR in the screening for dementia is by analyzing longitudinal score changes, as the baseline IQ affects most of the items in the DMR. Evenhuis' published criterion for a positive dementia screen on the basis of longitudinal score changes is either an increase of the SCS of 7 points or more and/or an increase of the SOS of 5 points or more over subsequent tests.

Manpower was not available to provide individual medical assessments and diagnosis of cognitive impairment and/or dementia. We therefore decided to use individual measures of yearly decline on the SCS and the SOS from scores on the first four detailed assessments for each participant in the study, using the least squares method [31]. This results in a separate slope for each individual representing change over time in each subscale. The formula used to derive the slope is shown as follows

$$\text{Slope}_i = \frac{\sum_{j=1}^{4}\left(x_{ij} - \overline{x_i}\right)\left(y_{ij} - \overline{y_i}\right)}{\sum_{j=1}^{4}\left(x_{ij} - \overline{x_i}\right)^2}. \quad (1)$$

In this equation, for n participants who had 4 tests each, y_{ij} represents the outcome for the ith participant at the jth time, and x_{ij} is the independent variable for the ith participant at the jth time. $\overline{y_i}$ represents the mean outcome for the ith participant, and $\overline{x_i}$ represents the mean value of the independent variable for the ith participant. We were aware that slopes derived with this method would capture only individual changes pooled over all of their first four assessments, rather than individual changes between specific assessments, and felt that this was a reasonable approach as individuals occasionally had fluctuations in their functioning in specific tests due to medical or social reasons.

2.3. The Reiss Screen for Maladaptive Behaviour (RSMB). The 38-item Reiss Screen for Maladaptive Behavior [29] was chosen to screen for depressive symptoms because it was a well-known, caregiver-rated scale for people with ID (not necessarily old), whose scores on its various subscales could be compared to normative data, and correlated with psychiatric syndromes of clinical interest (in this case depression). The eight core psychiatric subscales of the Reiss Scale include aggressive behavior, autism, psychosis, paranoia, depression (behavioral signs: anxious, crying spells, fearful, overly sensitive, sadness), depression (physical signs: body stress, eating problem, low energy, regressive behaviour, sleep problem), dependent personality disorder, and avoidant personality disorder. Items were initially designed to be completed by two separate caregivers who know the person well, and final scores on each category were to be based on the average of the two scores. In clinical practice, the Reiss Screen is frequently completed by one caregiver because of time constraints. Scores above the published cutoff scores for the individual subscales (aggression:5, autism:4, psychosis:5, paranoia:5, depression (behavioral signs):5, depression (physical signs):4, dependent personality disorder:6, and avoidant personality disorder:5) indicate clinical problems and the need for a further clinical assessment. Good psychometric properties were described by Reiss et al. in [29], although abnormal scores in subscales are clearly not analogous to standard clinical diagnoses. Some concerns have more recently been expressed about the characteristics of many screening instruments, including the Reiss Screen, in people with ID [32]. However, at the time this study was conceived, it was not possible to administer more detailed or comprehensive assessments.

2.4. Data Management and Analysis. Full data were collected from four formal data-assessment waves (1995-1996, 1997, 1999, and 2001), and limited data (mortality, nursing home placement) was collected from ongoing follow-up telephone surveys, most recently in July 2011. All data were entered by research assistants into a secure access database designed by the principal investigator, and data accuracy was verified for at least 25% of all data entries in each wave. Data in one wave was reentered due to greater than 5% errors. Descriptive results of the data were initially organized into tabular and graphic forms, exploring the patterns of univariate associations between baseline variables including age, sex, DS diagnosis, seizure history, and frequency of current seizures, health problems (including baseline caregiver identified confirmed or suspected dementia), baseline cognitive and psychiatric symptoms (from the DMR and the Reiss Screen), psychotropic medication use, IQ score, and mortality. IQ was dropped from the analysis because not enough valid scores were available in the sample. The baseline DMR practical skills subscale score was chosen as the main measure approximating the level of global deficits at entry to the study, acknowledging that this level of baseline skills would represent both baseline adult abilities as well as decrements from any preclinical degenerative processes. Level of ID (borderline, mild, moderate, severe, and profound) was also

dropped from the analysis because the interrater agreement across waves was low.

The remote history of seizures may not have been as accurate as information pertaining to seizures in the recent year, as there is known to be a high turnover in care staff, and many of our informants may have had mostly recent information about the participants. However, caregivers during the detailed assessments in waves 1 to 4 were asked to provide their best answers to the current and past presence of seizures based on all information available to them using the following rating: 0—never a history of seizures, 1—previously seizures but no seizures in the past year or more, 2—seizures occurring at the rate of less than one seizure per month, 3—one to four seizures per month, 4—two to six seizures per week, or 5—daily seizures. Seizures were explored in the regression analysis using a number of recoded variables: no seizures ever documented, seizures present at or before baseline, active seizures present at baseline, seizures reported in any of the four active data collection waves and seizures occurring during the followup but not at baseline (new seizures).

2.5. Statistical Analysis. Survival analysis was used to assess differential mortality during the course of the study. Participants were followed for a maximum of 16 years, with some (very few) leaving the study prematurely and some dying prior to the most recent contact in July 2011. Cox's proportional hazards modeling technique [33] was used to assess differential mortality, as it allows for the analysis of mortality rates based on different lengths of followup, adjusting for various independent variables in the regression model. It was not possible to do a time-dependent analysis for the independent variables as detailed data on most (such as medication use, occurrence of seizures) was only available for four waves of data collection, as described above. To compensate for this shortcoming, we used both the variable score at baseline and a recoded variable representing a pooled measure of the variable. For example, baseline use of antipsychotic medications was added into the model as well as a variable coding for the presence of an antipsychotic during any of the four detailed data collection waves. In the case of seizures, where the new onset of seizures is known to be associated with dementia in persons with DS, a third variable was created to represent people who developed new seizures after baseline.

Variables that were added to the initial Cox regression model using SPSS version 19 [34] included DS (0,1), sex (males = 1, females = 2), age (at baseline in years), number of years followed, deceased as of July 2011 (0,1), DMR practical skills subscale score (score 0–16), DMR Sum of Social Scores change per year and DMR Sum of Cognitive Scores individual change per year over waves 1–4, use of medications (antipsychotics, anxiolytics, antidepressants, sedative-hypnotics, anticonvulsants) at any of the four detailed waves of data collection, seizure status as described earlier, and the depression-related Reiss subscale scores at baseline (depression—behavioral, depression—physical).

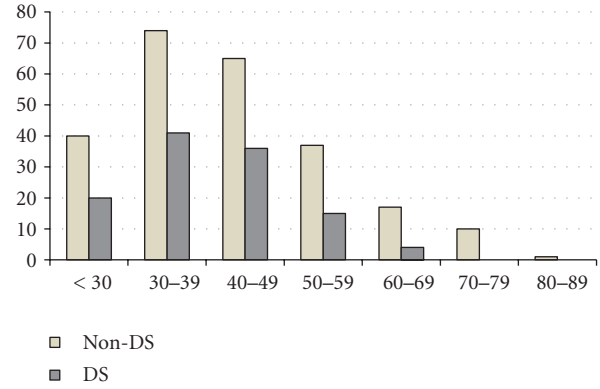

Figure 1: Age distribution of study participants at baseline (N = 360).

Core variables based on DS diagnosis, age, sex, and DS-Age interaction term were kept in every initial model regardless of statistical significance. Terms were then removed manually from the model in order of least statistical significance. Results are presented as hazard ratios and their 95% confidence intervals. The proportionality assumption was satisfied when tested using the log minus log test.

3. Results

Participants came from all areas of the province except for the far north, with the largest number originating from urban centres, consistent with the population distribution of the province. Participant living situations included Community Living Division group homes, private care homes, mental health approved homes, assisted living facilities, independent dwellings, family homes, and one larger congregate living site (but not the provincial institution for ID).

This study population represented a sizable proportion of the overall service population recorded by the Department of Social Services in 1995. In 1995, 3214 people with ID received services or 0.32% of the total population of Saskatchewan based on the 1996 census. Our participants represented 9.9%, 22.8%, 17.4%, and 9% of this ID service population within age groups 21–35, 36–54, 55–64, and 65+, respectively.

The 360 participants providing data between 1995 and 2011 included 142 non-DS males, 102 non-DS females, 64 DS males, and 52 DS females. More males than females entered the study (female to male ratio: 1 : 1.34), and the DS group was about three years younger on average than the non-DS group ($P < 0.05$ using independent samples t-test). Males and females were not significantly different in age. Basic demographics are shown in Table 1 and shown graphically in Figure 1.

Baseline scores on key variables entered into the initial model are shown in Tables 2 and 3.

At the most recent analysis in July 2011, 108 participants had died, and 9 had withdrawn for various reasons, leaving 243 in active followup. Follow-up time varied from 0 (only

Table 1: Demographics of participants at entry to the study.

	Non-DS			DS			All diagnoses		
Age	Male	Female	Total	Male	Female	Total	Male	Female	Total
<30	24	16	40	13	7	20	37	23	60
30–39	42	32	74	22	19	41	64	51	115
40–49	39	26	65	19	17	36	58	43	101
50–59	21	16	37	8	7	15	29	23	52
60–69	7	10	17	2	2	4	9	12	21
70–79	8	2	10	0	0	0	8	2	10
80–89	1	0	1	0	0	0	1	0	1
Total	142	102	244	64	52	116	206	154	360
Mean	43.17	42.91	43.06	39.73	40.48	40.07	42.1	42.09	42.09
SE	1.18	1.23	0.85	1.27	1.35	0.92	0.91	0.94	0.65
Range	17–83	20–71	17–83	20–61	20–61	20–61	17–83	20–71	17–83

Table 2: Summary of key variables entered into the survival model (categorical variables).

Variable	Variable detail	Non-DS		DS		Total	
Deceased	As of July 2011	66	27%	42	36.2%	108	30.0
Dementia	Caregiver reported at baseline	1	0.4%	6	5.2%	7	1.9
Medications	Antipsychotic at baseline	58	23.8%	37	31.9%	95	26.4
	Antipsychotic in any wave	83	34%	49	42.2%	132	36.7
	Antidepressant at baseline	30	12.3%	17	14.7%	47	13.1
	Antidepressant in any wave	56	23%	26	22.4%	82	22.8
	Sedative-hypnotic at baseline	5	2%	6	5.2%	11	3.1
	Sedative-hypnotic in any wave	24	9.8%	14	12.1%	38	10.6
	Anxiolytic at baseline	25	10.2%	11	9.5%	36	10.0
	Anxiolytic in any wave	50	20.5%	26	22.4%	76	21.1
	Anticonvulsant at baseline	66	27%	36	31%	102	28.3
	Anticonvulsant in any wave	81	33.2%	48	41.4%	129	35.8
Seizure history	Seizure history (current or past) at baseline	84	34.4%	14	12.1%	98	27.2
	Seizures (actively) present at baseline	39	16%	6	5.2%	45	12.5
	Seizures reported in any of the four waves	80	32.8%	46	39.7%	126	35
	New seizures reported after baseline	16	6.6%	12	10.3%	28	7.8

* Higher scores indicate greater deficits.
** Higher scores indicate greater yearly increase in deficits between 1995 and 2001.

one assessment before leaving the study for any reason) to 16.1 years as of July 2011, with the mean of 12.93 (0.21) years (Figure 2).

The number and percentages of deceased participants and mean ages of death and various categories are shown in Table 4.

Based on Cox proportional hazards models with the pooled DS and non-DS participants, leaving in the almost significant ($P = 0.08$) DS-age interaction term, sex, age at baseline, baseline practical skills deficits, baseline depression symptoms (Reiss behavioural depression), yearly decline on DMR social skills, a seizure history at baseline, a seizure history at any point before and during the study, and anticonvulsant use at baseline were all independently statistically significant to the prediction of mortality, as shown in Table 5. The derived seizure variable, new seizure, representing seizures arising after the beginning of the study, was not significant to mortality prediction. The use (baseline or during any of the first four waves) of psychotropic medications including antipsychotics, antidepressants, anxiolytics, and sedative-hypnotics were also not significant predictors of mortality. Also not significant was the caregiver designation of suspected or confirmed dementia or cognitive impairment at baseline.

A separate survival analysis (adjusting for DS, age, and sex) was performed to explore whether the use of an anticonvulsant during any of the first four detailed data collection waves in the absence of a current or previous history of seizures increased mortality. Although there was a trend supporting this, it was not significant ($P = 0.26$). Similarly, a separate survival analysis (adjusting for DS, age, and sex) was conducted to explore the possibility that the

Table 3: Summary of key variables entered into the survival model (continuous variables).

Variable	Variable detail	Non-DS		DS		Total	
		Mean (SE)	Range	Mean (SE)	Range	Mean (SE)	Range
Years followed	As of July 2011	13.26 (0.25)	0.59–16.10	12.26 (0.39)	0.0–16.05	12.93 (0.21)	0–16.10
Age	Baseline	43.06 (0.85)	17–83	40.07 (0.92)	20–61	42.08 (0.66)	17–83
DMR (baseline)*	Practical skills subscale score	2.20 (0.24)	0–16	0.98 (0.22)	0–16	1.81 (0.18)	0–16
Reiss Screen baseline	Depression (Behavioral)	1.30 (0.11)	0–8	0.93 (0.13)	0–7	1.18 (0.08)	0–8
	Depression (Physical)	1.33 (0.10)	0–6	1.44 (0.16)	0–7	1.36 (0.09)	0–7
DMR change per year**	Sum of Cognitive Scores (SCS)	0.33 (0.11)	−5.26–13.14	0.71 (0.18)	−2.12–11.99	0.45 (0.10)	−5.26–13.14
DMR change per year**	Sum of Social Scores (SOS)	0.50 (0.12)	−3.92–8.34	0.82 (0.23)	−6.84–11.60	0.61 (0.11)	−6.84 −11.60

* Higher scores indicate greater deficits.
** Higher scores indicate greater yearly increase in deficits between 1995 and 2001.

Table 4: Number (%) of the baseline cohort deceased and the mean age of death as of July 2011.

Sex	Non-DS		DS		All	
	Number (%) deceased	Age of death (SE)	Number (%) deceased	Age of death (SE)	Number (%) deceased	Age of death (SE)
Males	43 (30.3)	56.0 (2.0)	23 (35.9)	61.7 (2.6)	65 (31.9)	59.7 (1.6)
Females	29 (28.4)	61.6 (1.8)	13 (25.0)	60.1 (3.1)	43 (27.6)	58.6 (1.7)
All	66 (27.0)	61.1 (1.7)	42 (36.2)	56.3 (1.3)	108 (30.0)	59.2 (1.2)

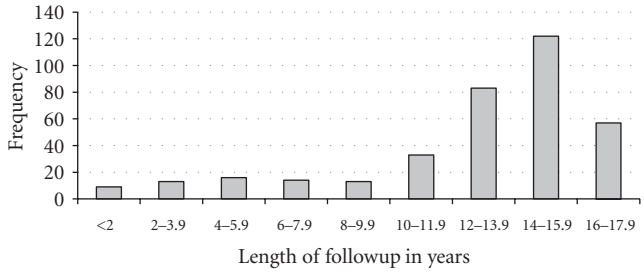

Figure 2: Length of follow-up of study participants by July 2011.

use of an antipsychotic in the absence of a clinical diagnosis reported by a caregiver of a mental health problem for which the use of an antipsychotic is generally appropriate (any psychotic disorder such as schizophrenia or delusional disorder, or bipolar disorder) might increase mortality. This was also negative ($P = 0.25$).

Because people with DS are known to have shorter lifespans and higher risks of dementia, even though the DS-Age interaction was not quite significant, the following analyses were repeated with the groups stratified by DS. Table 6 shows that mortality in people without DS was predicted by increased age, higher levels of Reiss physical depression scores, greater decline in DMR social scores per year during the first four waves, and a seizure history at or before baseline. Unlike in the pooled DS and non-DS analysis, sex, baseline practical skills deficits, baseline anticonvulsant use, baseline Reiss behavioral depression, and seizure at any time before or during the study were not significant predictors of mortality.

Cox regression for mortality in participants with DS resulted in a different model. Table 7 shows that mortality in people with DS was increased by male sex, older baseline age, increased baseline practical skills deficits, higher levels of baseline Reiss behavioural depression scores, and greater decline in DMR cognitive scores per year during the first four waves.

Contrasts between some of the survival curves (adjusted as shown in Tables 6 and 7) for participants with and without DS are illustrated in Figures 3, 4, and 5.

4. Discussion

4.1. Pooled DS and Non-DS Analysis. Based on our pooled DS and non-DS analysis (including the almost significant DS ∗ age interaction term), some of our findings regarding the prediction of increased mortality, such as the presence of DS, older age, and lower baseline level of baseline functioning, do not challenge the general understanding about mortality in ID. However, we did not find that males with DS had any special protection compared to females, unlike some findings reported by others (cited earlier). We instead found that males, just as in the general population, had increased mortality when findings were adjusted for the other significant predictors including age and baseline functioning.

We had not expected any impact of baseline depressive symptoms on the eventual mortality when the study was

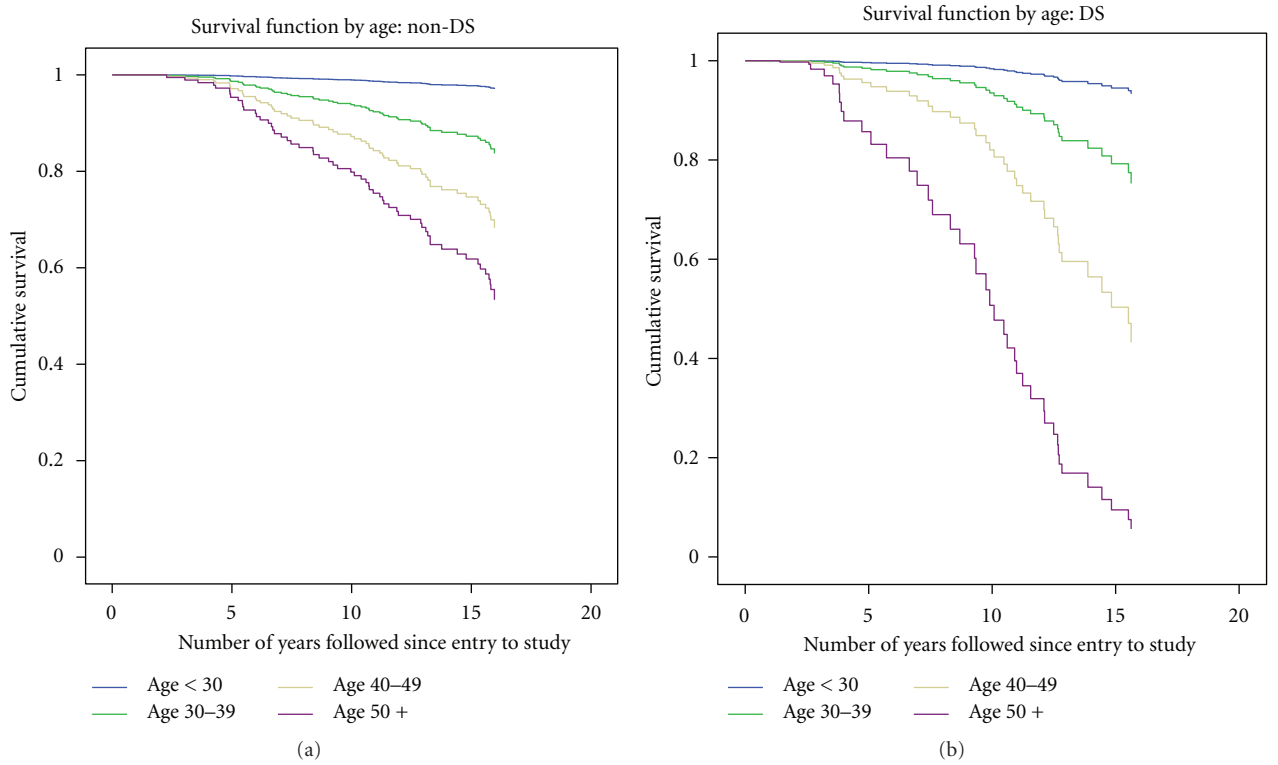

Figure 3: Adjusted survival curves of 360 participants with ID (1995–2011): impact of baseline age: non-DS compared to DS.

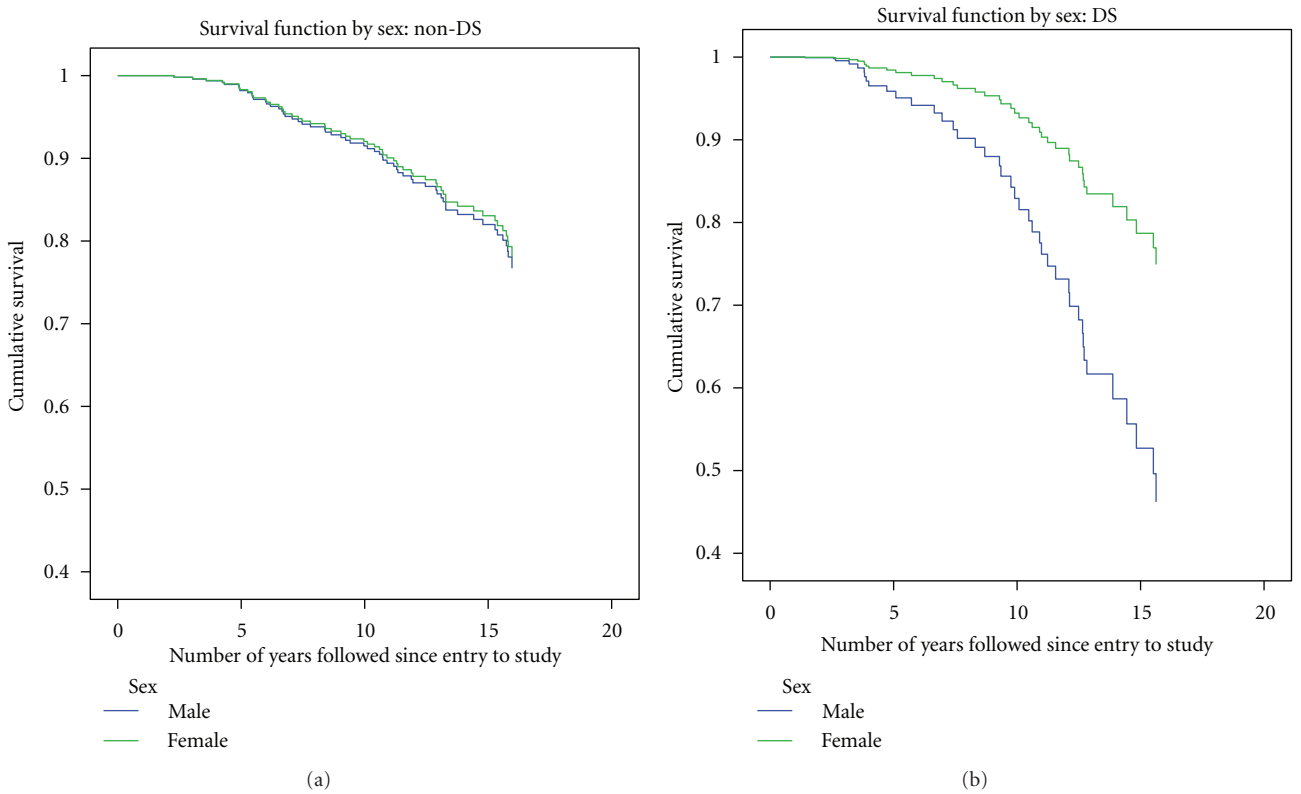

Figure 4: Adjusted survival curves of 360 participants with ID (1995–2011): impact of sex: non-DS compared to DS.

Table 5: Multivariate Cox regression analysis of mortality, as of July 2011.

	β (SE)	Sig.	Hazard ratio (95% CI)
Down Syndrome	−0.34 (1.00)	NS	0.10–5.11
Sex (ref: male)	−0.58 (0.23)	<0.05	0.36–0.88
Baseline age	0.06 (0.01)	<0.0001	1.05–1.09
Baseline DMR practical skills deficits	0.09 (0.03)	<0.005	1.03–1.16
Baseline Reiss behavioral depression	0.25 (0.06)	<0.0005	1.12–1.44
Baseline anticonvulsant use	0.71 (0.33)	<0.05	1.07–3.90
DMR Sum of Social Scores change per year	0.27 (0.05)	<0.0001	1.18–1.45
History of seizure at or before baseline	0.50 (0.24)	<0.05	1.04–2.63
Seizure before or during the study	−0.69 (0.31)	<0.05	0.28–0.91
Baseline age ∗ DS interaction	0.04 (0.02)	0.08	1.00–1.08

Table 6: Multivariate Cox regression analysis of mortality, as of July 2011 (Non-DS).

	β (SE)	Sig.	Hazard ratio (95% CI)
Baseline age	0.06 (0.01)	<0.0001	1.04–1.08
Baseline Reiss physical depression	0.19 (0.08)	<0.05	1.03–1.41
DMR Sum of Social Scores change per year	0.34 (0.08)	<0.0001	1.21–1.63
History of seizure at or before baseline	0.59 (0.28)	<0.05	1.05–3.09

designed, and this association was found only after comprehensive exploration of all valid baseline variables in the Cox regression analysis. The ID population is not known to have high suicide rates, and especially in supportive settings in our community their level of preventive health care (and rates of smoking) is likely better than that in the general population. For example, most of our local community group homes have excellent policies regarding yearly medical assessments and screening. Self-harm attempts in people with depression related to driving (inattention, purposeful risk-taking) are also very unlikely in ID. The association between baseline depressive symptoms and increased mortality in our study is therefore consistent with previously cited data suggesting that some other factors, such as inactivity, autonomic dysfunction, and inflammation, may have an important role to play. It is also possible that depressive symptoms are a marker for early dementia, which is independently related to increased mortality. However, the risk remained even when adjusted for by yearly decline in social scores, as well as caregiver direct reports of dementia or other cognitive functional decline, so it would appear that depressive symptoms may still have an additive adverse impact.

In our study, the use of antidepressants at baseline or at any point in the first four data collection waves did not contribute significantly in either direction to mortality. Unfortunately, our data did not allow us to fully explore whether the treatment of depression with antidepressants (using a time covarying analytic technique) improved this increased mortality risk, as we did not have data on antidepressant use for the entire study period. This would have been more ideal, as the data on treatment of depression and mortality is contradictory, with some recent research, such as that from the Women's Health Initiative Study [35] even suggesting an increased mortality and stroke risk in women on these mediations.

The baseline use of anticonvulsants was associated with increased mortality in our sample, even when adjusted for seizures present at or before baseline and seizures present at any time during the study. Anticonvulsants have many adverse effects, including drug-drug interactions, which may have played a role. It would have been ideal to have detailed information about the time of the original onset of seizures, as this might have been significant to eventual mortality outcome. Although we did not find that there was an additional adverse impact on mortality by the use of anticonvulsants in the absence of seizures, the potential red flag of the use of anticonvulsants suggests increased caution in using these drugs, especially for behavioral reasons, where alternate interventions might be instituted.

The presence of a seizure before or at baseline independently increased mortality risk ($P < 0.05$), as did the presence of a seizure at any point during the study ($P < 0.05$). The significance of both of these predictors independently in the model may suggest that both an early onset of seizures and later seizures may have different mechanisms of action accounting for their association with increased mortality, but result in increased cumulative burden. For example, early onset epilepsy is associated in sudden unexplained death (cited earlier), and is also associated with other physical problems which may increase mortality, whereas later onset of seizures, especially in those with DS, is associated with the development of dementia, itself a predictor of increased mortality.

We did not find that the baseline use (or use during any of the first four waves of detailed data collection) of antipsychotics contributed to mortality, unlike some studies in frail,

Table 7: Multivariate Cox regression analysis of mortality, as of July 2011 (DS).

	β (SE)	Sig.	Hazard ratio (95% CI)
Sex (ref: male)	−0.98 (0.35)	<0.01	0.187–0.75
Baseline age	0.10 (0.02)	<0.0001	1.06–1.15
Baseline DMR practical skills deficits	0.21 (0.07)	<0.005	1.07–1.41
Baseline Reiss behavioral depression	0.27 (0.11)	<0.01	1.07–1.62
DMR Sum of Cognitive Scores change per year	0.32 (0.08)	<0.0005	1.17–1.63

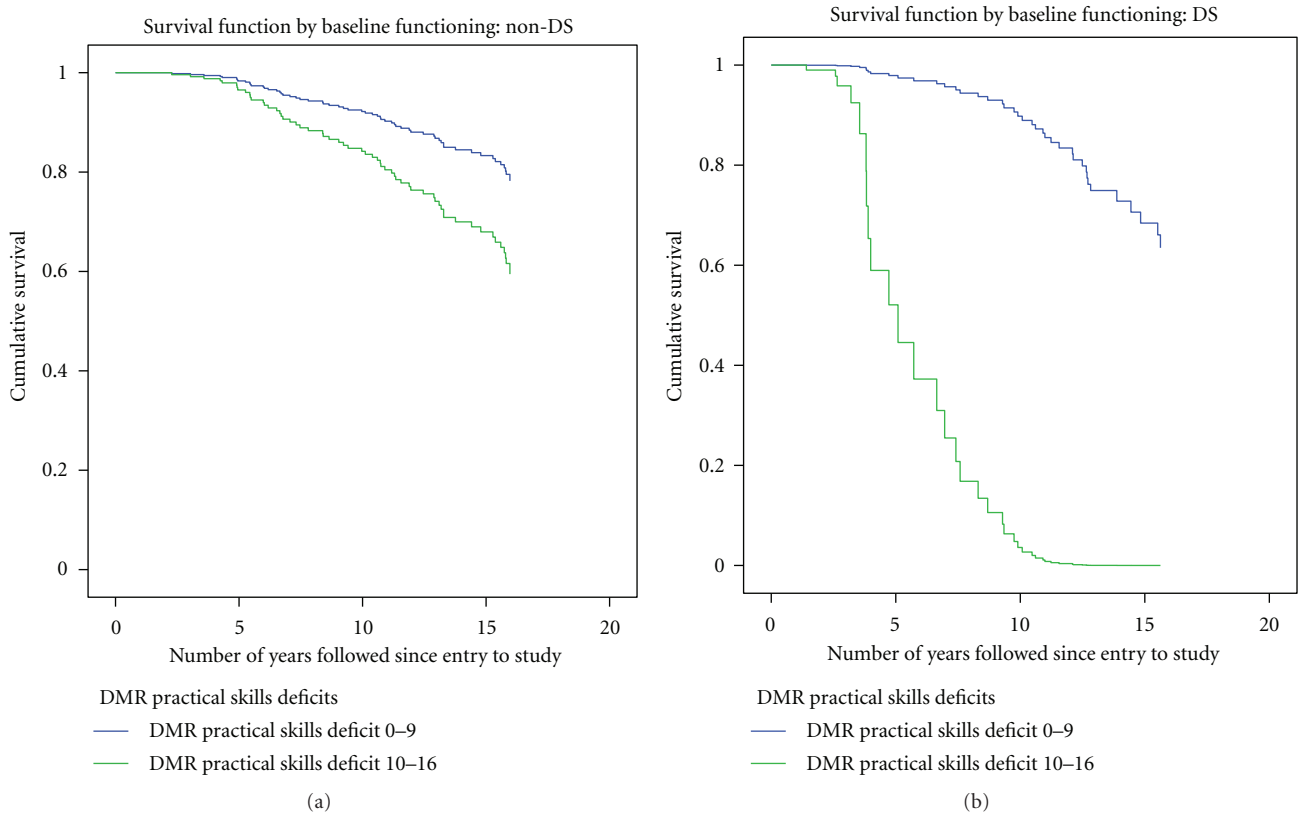

Figure 5: Adjusted survival curves of 360 participants with ID (1995–2011): impact of baseline functional deficits: non-DS compared to DS.

demented people without ID. It is possible that healthier, strong, people with ID may be more likely to exhibit risk to others from aggressive behaviours and are therefore more likely to be prescribed these agents. The prescription of antipsychotics then might be a marker for decreased mortality at baseline, masking any other direct adverse effects. We also did not find an increased adverse impact of the use of antipsychotics in the absence of a diagnosis of a psychotic or bipolar disorder, for which antipsychotic use is frequently indicated. Unfortunately, these diagnoses were obtained from caregivers and chart information rather than individual, standardized assessments, so their validity may be questionable.

Greater individual yearly changes in the DMR Sum of Social Scores over the first four data collection waves were associated with increased mortality, even when adjusted for by age and other factors. Although the yearly changes were very small in all groups, this association suggests that, even in young people, subtle decline in functioning might be predictive of later, poor outcomes.

4.2. Stratified DS and Non-DS Analysis. Stratification of the analysis into participants with and without DS had face value in light of the aging differences well established by others, although the reduction in numbers likely resulted in the loss of ability to find significance in some of the potentially predictive factors for mortality. For example, the use of anticonvulsants was no longer statistically significant to outcome in either group, sex was not significant to mortality in those without DS, and the impact of seizures

in those with DS (low numbers) could not be ascertained. However, some patterns more specific to DS likely emerged from this approach. Baseline practical skills deficits were strong predictors of mortality in DS but not in those without DS, even when adjusted for by age, clinical diagnosis of dementia, and other significant predictive variables. This difference could have arisen because participants with DS may have had their baseline functioning already impaired by early cognitive decline which was not recognized by caregivers, and this decline itself increased mortality. Also differing between the DS and non-DS group was the type of depressive symptoms found to be significant predictors of mortality. Baseline physical (rather than behavioral) symptoms of depression including body stress, eating problems, low energy, regressive behaviour, and sleep problem were predictive of increased mortality in participants without DS. In contrast, in participants with DS, behavioural symptoms of depression (anxious, crying spells, fearful, overly sensitive, sadness) were found to be significant predictors on increased mortality. The reason for this discrepancy is not readily apparent.

4.3. Study Limitations and Summary. Our study was limited by small sample size, lack of sophisticated imaging, lack of detailed data on causes of death, lack of medication and seizure data throughout the whole study, and lack of individualized and standardized clinical diagnosis. In spite of this, the long follow-up time may provide valuable insights into baseline predictors of serious health outcomes, and may result in further improvements to life expectancy, especially for those with higher mortalities or higher rates of depressive symptoms. In particular, clinicians should take depressive symptoms very seriously, evaluating associated health issues and carefully the necessity for further consultation with specialty services. The use of anticonvulsants for reasons (such as behavioural problems) other than epilepsy should be considered carefully, and perhaps only instituted if there is a lack of response to other interventions. The presence of seizures (early onset and later onset) is always a risk for adverse health outcomes, yet excessive vigilance may also result in decreased autonomous functioning and resultant quality of life.

Disclosure

This is an original publication produced by the authors. Data presented in figures was derived from the Intellectual Disability and Aging Study, approved by the University of Saskatchewan Ethics Committee.

Authors' Contribution

Dr. Thorpe conceived of the study, supervised data collection, carried out analysis and wrote the initial drafts of the paper. Drs. Bennett, Kirk and Nanson helped develop the methodology of the study at its conception, and collaborated in editing the paper. Dr. Pahwa helped with the statistical analysis and also collaborated in editing the paper.

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Research Article

Sterol Lipid Metabolism in Down Syndrome Revisited: Down Syndrome Is Associated with a Selective Reduction in Serum Brassicasterol Levels

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Over the past 15 years, insights into sterol metabolism have improved our understanding of the relationship between lipids and common conditions such as atherosclerosis and Alzheimer's Disease (AD). A better understanding of sterol lipid metabolism in individuals with Down Syndrome (DS) may help elucidate how this population's unique metabolic characteristics influence their risks for atherosclerosis and AD. To revisit the question of whether sterol lipid parameters may be altered in DS subjects, we performed a pilot study to assess traditional serum sterol lipids and lipoproteins, as well as markers of sterol biosynthesis, metabolites, and plant sterols in 20 subjects with DS compared to age-matched controls. Here we report that the levels of nearly all lipids and lipoproteins examined are similar to control subjects, suggesting that trisomy 21 does not lead to pronounced general alterations in sterol lipid metabolism. However, the levels of serum brassicasterol were markedly reduced in DS subjects.

1. Introduction

1.1. Down Syndrome. Down Syndrome (trisomy 21) is the most common chromosomal abnormality, occurring in approximately 1 in 800 live births. DS is characterized by typical dysmorphic features, congenital abnormalities, and other medical conditions. Over the past 15 years, the life expectancy of individuals with DS has increased significantly, with the median age of death currently approaching 50 years [1], an age where the incidence of many common diseases of aging is high. Importantly, there are several differences in the way individuals with DS appear to age compared to the general population. Chief among these is the inevitable appearance of Alzheimer's Disease (AD) neuropathology by the age of 35 years [2]. Individuals with DS have also been reported to be relatively resistant to developing atherosclerosis despite the presence of an unfavorable plasma lipid profile [3]. AD and atherosclerosis are each complex, multifactorial diseases with both genetic and environmental contributors [4, 5]. As lipid metabolism contributes to the pathogenesis of both disorders [4, 5], studying lipid metabolic markers in the unique clinical situation of DS may allow our understanding of the pathogenesis and risk factors of these diseases to be refined for both the DS and the general populations.

1.2. Atherosclerosis in DS. Since Murdoch described a complete lack of atheroma in five institutionalized people with DS, there has been considerable interest in DS as an "atheroma-free" model [6]. Two subsequent post-mortem studies also demonstrated lower atheroma burden in institutionalized individuals with DS compared to age-matched controls [7, 8]. A recent study demonstrated reduced intima-media thickness in the carotid arteries of community-dwelling individuals with DS [9], which helped to address criticisms over the institutionalized populations used in the

previous reports. These findings are particularly striking in light of the fact that individuals with mental retardation are typically at increased risk for atherosclerosis [10]. Indeed, the hypothesis that individuals with DS are protected from the development of atherosclerosis is interesting, but an explanation for this observation has not been elucidated to date.

Atherosclerosis is a complex, progressive inflammatory disorder in which dysregulated lipid metabolism plays a central role [5]. The causal link between circulating cholesterol levels and atherosclerosis is well established. For example, elevated levels of low-density lipoprotein cholesterol (LDL-C) definitively increase atherosclerosis risk [11, 12]. LDL, which transports cholesterol from the liver to peripheral tissues, satisfies all of Koch's modified postulates and has a causal role in the pathogenesis of atherosclerosis [13]. This role is best illustrated by the success of statins and other cholesterol lowering medications in reducing LDL-C levels, thereby decreasing the number of cardiovascular events in treated patients [14]. Not surprisingly, however, given the complexity of atherosclerotic disease, lipoproteins other than LDL may also contribute.

High-density lipoprotein (HDL) is the plasma lipoprotein that mediates reverse cholesterol transport, a process that extracts excess cholesterol from peripheral tissues and transports it to the liver to be ultimately excreted as bile [15]. Elevated levels of HDL-C have been clearly shown to be protective against the development of atherosclerosis even in the context of high LDL-C levels [11, 16]. Through intense investigations on HDL biogenesis and function, several members of the ATP binding cassette (ABC) superfamily have been characterized. ABCA1 and ABCG1 are genes that encode for proteins involved in the efflux of cholesterol from peripheral cells onto HDL [17]. ABCA1 catalyses the initial transfer of lipids onto apolipoprotein A-I (apoA-I), which is the rate-limiting step in the formation of nascent HDL particles [18]. ABCG1 continues this process of adding lipids to HDL [18]. Notably, ABCG1 localizes to the long arm of chromosome 21 [19] and is inherited in triplicate in most people with DS, raising interesting questions about whether excess ABCG1 may underlie some of the differences in lipid metabolism in this group compared to the typically developing population. Intriguing new data from preclinical studies show that ABCG1 also has important roles in endothelial function, where it promotes oxysterol efflux and protects from hypercholesterolemia-mediated endothelial dysfunction [20]. Conversely, genetic deficiency of ABCG1 in mice promotes endothelial activation, enhances monocyte adhesion and increases vascular inflammation [21]. Although these mechanisms have yet to be examined in DS subjects, abundant ABCG1 function in the endothelium may help to explain their relative protection from atherosclerosis.

Studies of plant sterols have further expanded our knowledge of the role of lipids in the progression of atherosclerosis. Because a high intake of plant sterols reduces circulating cholesterol levels [22], functional foods enriched in plant sterols are now offered commercially as a means to lower total plasma cholesterol levels. Plant sterols differ from cholesterol by the presence of a double bond at C22 and/or a methyl or ethyl group at position C24 [22]. The major plants sterols include campesterol (methyl C24), sitosterol, (ethyl C24), brassicasterol (D22, methyl C24), and stigmasterol (D22, ethyl 24). In the enterocyte, plant sterols are believed to compete with cholesterol for incorporation into micelles, thereby reducing cholesterol absorption [22]. Several lines of evidence suggest that increased levels of circulating plant sterols correspond with a decreased risk for cardiovascular disease. Ntanios et al. have shown hamsters fed a diet enriched in phytosterol esters have significantly fewer foam cells, suggesting that phytosterols inhibit a key step in the progression of atherosclerosis [23]. Fassbender et al. have also shown an association between elevated circulating plant sterols and a reduced tendency towards symptomatic atherosclerosis in a Dutch cohort [24].

However, other observations complicate this issue, the most notable of which is that sitosterolemic patients, who have markedly increased circulating sitosterol levels, exhibit accelerated atherogenesis [25]. Sitosterolemia is a rare autosomal recessive condition caused by mutations in two other ABC transporters: ABCG5, and ABCG8 [26]. Both ABCG5 and ABCG8 are expressed exclusively in the enterocyte and efflux plant sterols from the enterocyte back into the intestinal lumen, thereby reducing plant sterol absorption [27]. In sitosterolemia, lack of ABCG5/ABCG8 function leads to significantly greater absorption of dietary cholesterol and sitosterol and an increased incidence of cardiovascular events independent of plasma LDL-C levels, suggesting that sitosterol itself may be contributing to atheroma formation [25]. A recent *in vitro* study also showed differential effects of various plant sterols, both protective and deleterious, on ABC transporter expression in foam cells [28]. There is currently no clear consensus on the contributions of plant sterols to the development of atherosclerosis, but recent research suggests that numerous pathways may be involved. It is not yet known whether plasma levels of plant sterols in people with DS differ from the general population.

In attempts to better understand the pathogenesis of atherosclerosis in individuals with DS, several groups have investigated the traditional atherosclerotic risk factors of circulating total cholesterol (TC), LDL-C, and HDL-C in the DS population. Although these studies all vary significantly in their sample sizes, specific outcomes, and control groups, they nonetheless provide some useful insights into the traditional atherosclerotic risk profiles of the DS population. One study demonstrated a favorable lipid profile in individuals with DS, yet noted elevated levels of homocysteine, which has been suggested to increase atherosclerosis risk [29]. Several studies found no change in serum LDL-C or HDL-C in individuals with DS compared to a control group or to population norms [9, 30–32]. Many other studies demonstrated an increased number of atherosclerotic risk factors in individuals with DS. For example, Draheim et al. observed elevated triglycerides and total body fat in their DS study group [31]. These findings agree with a much earlier study by Nishida et al. [32] and are consistent with our current understanding of the metabolic syndrome, which is common in DS. Other studies have also observed a lipid profile that would suggest individuals with DS to

be at an increased risk for atherosclerosis [29, 33]. These widely variable results underscore the lack of consensus as to whether lipid profiles of individuals with DS vary from the general population and whether they confer increased atherosclerotic risk. Notably, factors known to correlate with atherosclerosis in the general population, such as fruit and vegetable intake, serum LDL-C, and smoking status, all correlate poorly with intima-media thickness in individuals with DS [9]. This surprising result, in addition to the conflicting data surrounding traditional atherosclerotic risk factors, suggests that there may be some distinct mechanisms underlying atheroma formation in DS.

1.3. Alzheimer's Disease in DS. AD is the most common form of dementia in the elderly and currently affects over 50% of the general population greater than 85 years of age [4]. The vast majority (96%) of AD patients begin to experience memory dysfunction in their 60s–80s [4]. The remaining patients carry genetic mutations that lead to an early-onset familial form of AD, which can manifest as early as the mid 30s [34]. All AD patients develop two neuropathological hallmarks including amyloid plaques that consist of aggregated Aβ peptides and neurofibrillary tangles that contain hyperphosphorylated tau protein [35]. As detailed below, the 2011 guidelines for the diagnosis of AD recognize that changes in Aβ and tau metabolism begin to occur decades earlier than the onset of cognitive dysfunction [36].

It is well established that DS subjects inevitably develop amyloid and tau deposits by their mid 30s [37]. As amyloid precursor protein (APP), the protein from which Aβ peptides are derived, is on chromosome 21, inheritance of excess APP has been thought to underlie the accelerated onset of AD in the DS population although this is not universally accepted [38–41]. However, additional other mechanisms, many of which are related to lipid metabolism, may also contribute.

First, the levels and distribution of intracellular cholesterol can affect several aspects of APP and Aβ metabolism. For example, the proteolytic processing of APP into Aβ is highly influenced by intracellular cholesterol levels such that excess cholesterol increases Aβ production whereas cholesterol depletion minimizes it [42–45]. Once produced, Aβ peptides are degraded, often within microglia, and recent studies show that excess intracellular cholesterol in microglia delays Aβ degradation whereas cholesterol efflux from microglia promotes Aβ degradation [46].

Second, apolipoprotein E (apoE) is one of the protein components of chylomicrons and very low-density lipoproteins in plasma, as well as the major apolipoprotein in the brain [47]. The human APOE gene encodes three alleles, apoE2, apoE3, and apoE4. In 1993, apoE genotype was identified as a genetic risk factor for late onset AD [48, 49] and to this day remains the most robust genetic risk factor for late onset AD in the general population [50]. Although exactly how apoE4 contributes to increased AD risk is not entirely understood, apoE is known to bind Aβ and facilitates its proteolytic degradation, with apoE4 as less efficient in Aβ clearance than either apoE2 or apoE3 [46]. Increasing the lipid content on apoE facilitates Aβ clearance both *in vitro* and *in vivo* [51–55].

Third, epidemiological evidence suggests that plasma lipid levels are associated with AD risk. Specifically, high LDL-C and/or low HDL-C levels, particularly in midlife, have repeatedly been associated with AD risk [56–60]. Understanding the association between circulating cholesterol levels and AD risk has been challenging because neither cholesterol nor apoE crosses the blood brain barrier (BBB) [61]. However, apoA-I, the major apolipoprotein on HDL, is capable of BBB transit and has recently been shown to markedly affect cerebrovascular amyloid levels and cognitive function in mouse models of AD [62, 63]. Recently, lipidomic approaches suggest that decreased plasma sphingomyelin and increased plasma ceramide mass correlate with cognitive function in AD [64].

Finally, retrospective epidemiological studies suggest that statins, drugs that inhibit the rate-limiting step in cholesterol biosynthesis and are widely used to lower LDL-C levels, reduce AD prevalence [65–67]. Although subsequent prospective, randomized-controlled trials of statins failed to show efficacy in the ability of statins to either prevent or treat AD [68, 69], these trials, like many in the AD field, may have failed because treatment was initiated past the therapeutic window.

Identifying molecular and biochemical changes of AD early in the disease process is necessary to allow treatment to begin prior to neuronal loss and cognitive decline. To better define this therapeutic window, in 2011 the National Institutes of Health (NIH) released new clinical guidelines for the diagnosis of AD that incorporate the current understanding of early stages in AD pathogenesis [36]. The first detectable changes associated with AD are alterations in specific proteins in the cerebrospinal fluid (CSF), specifically decreased levels of Aβ 1–42 and increased levels of phosphorylated tau protein. This is followed by development of amyloid deposits in the brain, which can be visualized in a living patient using positron emission topography (PET) with a specific amyloid ligand known as Pittsburgh compound B (PIB). Neuronal atrophy, which follows a distinct pattern, later becomes detectable by magnetic resonance imaging (MRI). Finally, cognitive problems emerge. The sequence of AD pathology can begin up to 20 years prior to the onset of cognitive symptoms [70]. Clearly, these new guidelines represent an enormous advance on our ability to track the onset and progression of AD in the general population.

Very recently, Vanmierlo et al. demonstrated that the levels of the plant sterol brassicasterol were significantly reduced in the CSF of cognitively impaired AD subjects with an intact BBB [71]. Because plasma brassicasterol levels were unchanged, reduced CSF brassicasterol levels were hypothesized to reflect altered choroid plexus function during the progression of AD [71]. Importantly, in this study, CSF brassicasterol levels improved the predictive power of the other validated CSF biomarkers, Aβ and tau. Although their study was not designed to determine whether CSF brassicasterol levels may be prognostic of AD progression, their observations nonetheless generate interesting hypotheses about the utility of plant sterol metabolism as a potential biomarker in AD.

2. Study Design

2.1. Subjects. Twenty community-dwelling subjects with DS were assessed in this pilot study (Table 1). DS subjects were identified from the University of Irvine California clinic or group homes, all had clinical features of trisomy 21 and nearly all had karyotypic analyses confirming trisomy 21. The 22 typically developing control subjects did not have trisomy 21 and were excluded for AD, diabetes and obesity. Clinical Research Ethics Boards from the University of Irvine, California and the University of British Columbia approved this pilot study. Written informed consent was obtained from each DS subject or caregiver and control subject.

2.2. Plasma Lipid, Lipoprotein, and CRP Analysis. TC and HDL-C were measured from nonfasting serum by enzymatic kits (Wako) according to the manufacturer's protocols. ApoA-I and apolipoprotein B100 (apoB) were measured using an immune-nephelometric assay on the Siemens ProSpec automated analyzer (Siemens Diagnostics, Tarrytown, NY). The maximum interassay coefficient of variation (CV) of the assay is 2.2% and 1.9% for apoA-I and apoB, respectively. CRP was measured with a enzymatic chemiluminescent immunometric assay using the Siemens IMMULITE 2500 automated analyzer. The linear range of the assay is 0.2–150 mg/L, with a maximum interassay CV of 8.7%.

2.3. Sterol Extraction and Analysis from Plasma. Samples were frozen in aliquots and stored at −80°C until analysis. Serum concentrations of cholesterol were measured by gas chromatography-flame ionization detection using 5α-cholestane as internal standard. The cholesterol precursors lanosterol, dihydrolanosterol, lathosterol, and desmosterol, the plant sterols campesterol, brassicasterol, sitosterol, and stigmasterol as well as the 5α-saturated compounds cholestanol, campestanol and sitostanol were measured by a modified sensitive method using combined gas chromatography-mass spectrometry (GC-MS) using epicoprostanol as internal standard. The cholesterol oxidation products, 7α-, 24S-, and 27-hydroxycholesterol, were measured by GC-MS isotope dilution methodology using deuterium, that is, stable isotope labeled 7α-, 24R, S-, and 27-hydroxycholesterol as internal standards [72].

Fifty μg 5α-cholestane (Serva) (50 μL from a stock solution of 5α-cholestane in cyclohexane; 1 mg/mL) and 1 μg epicoprostanol (Sigma) (10 μL from a stock solution epicoprostanol in cyclohexane; 100 μg/mL) were added to 100 μL serum. One mL NaOH (1 M) in 80% ethanol was added and the alkaline hydrolysis was performed for 60 min at 61°C. The sterols were subsequently extracted with 3 mL of cyclohexane twice. The organic solvents were evaporated and the residual plasma sterols were dissolved in 160 μL n-decane. Eighty μL of the serum n-decane samples were transferred into microvials for gas-liquid chromatography-mass spectrometry—selected ion monitoring (GC-MS) of sterols, stanols and oxysterols. The sterols and stanols were derivatized to trimethylsilyl (TMSi) ethers by adding 10 μL TMSi-reagent (pyridine : hexamethyldisilazane-trimethylchlorosilane; 9 : 3 : 1, by volume; all reagents were applied from Merck) and incubated for 1 h at 64°C.

The residual 80 μL of the serum n-decane samples were diluted with 300 μL n-decane and derivatized with 30 μL TMSi-reagent preceding analysis of cholesterol by gas chromatography-flame ionization detection (GC-FID).

2.4. GC-FID and GC-MS. Plasma cholesterol was quantified by GC-FID on an HP 6890 series II plus GC (Agilent Technologies, Böblingen, Germany) using 5α-cholestane as an internal standard. An aliquot of 2 μL was injected in a splitless mode at 280°C by an automated sampler and injector (HP 7683). Hydrogen was used as carrier gas with an inlet pressure of 9.9 psi, resulting in a total gas-flow of 1.1 mL/min and the temperature of the flame ionization detector was kept at 280°C. The sterols were separated on a cross-linked methyl silicone DB-XLB 122-1232 fused silica capillary column (J&W, Folsom, USA) (30 m × 0.25 mm i.e., × 0.25 μm film thickness) in an Hewlett-Packard (HP 6890) gas chromatograph. The oven temperature was initially kept at 150°C for 3 min, and then gradually increased to a final temperature of 290°C. The ratios of the cholesterol areas to the area of internal standard were calculated and multiplied by the added amount of the internal standard (50 μg 5α-cholestane) to reveal absolute cholesterol concentrations.

GC-MS was performed on an HP GC-MSD system (HP 5890 series II GC) combined with a 5971 mass selective detector (Agilent Technologies, Böblingen, Germany) equipped with a DB-XLB 122-1232 fused silica capillary column (J&W, Folsom, USA) (30 m × 0.25 mm i.e., × 0.25 μm film thickness) in the splitless mode using helium (1 mL/min) as the carrier gas. The temperature program was as follows: 150°C for 1 min, followed by 20°C/min up to 260°C, and 10°C/min up to 280°C (for 15 min). The sterols, stanols, and oxysterols were monitored as their TMSi derivatives in the selected ion monitoring mode using their characteristic masses [73]. Identity of all sterols was proven by comparison with the full-scan mass spectra of authentic compounds (range, m/z 50–500). All the above sterols, stanols, and oxysterols were sufficiently separated on the column from each other. Accuracy of the method was established by recovery experiments, day to day variation (below 3%), limit of detection and limit of quantification below the present concentrations for each sterol.

2.5. Statistics. Data were analysed by unpaired two-tailed Student's t-test (GraphPad Prism v5.0), applying Welch's correction when variances were significantly different between groups. A $P < 0.05$ was considered statistically significant.

3. Results

3.1. Study Subjects. A total of 20 community-dwelling DS and 22 healthy, typically developing control subjects were recruited for this pilot study. The complete cohort (Table 1) did not differ statistically in mean age ($P = 0.167$) despite a wider age range in the control compared to the DS cohort.

Table 1: Demographics of control and DS cohorts.

	Con < 45	DS < 45	Con > 45	DS > 45	Con	DS
N	13	6	9	14	22	20
% male	61	83	22	64	45	70
Mean age (range)	38.92 (29–44)	42.53 (39–44)	50.78 (46–61)	47.8 (45–49)	43.77 (29–61)	46.29 (39–49)

Table 2: Serum analytes in the total control and total DS cohorts. Analytes shown in bold are significantly different between control and DS groups by unpaired Student's *t*-test. Welch's correction was applied when variances were significantly different between groups.

	Con	DS	*P*
TC (mmol/L)	4.858 (±0.743)	4.925 (±1.123)	0.834
HDL-C (mmol/L)	1.591(±0.514)	1.412 (±0.271)	0.116
ApoA-I (mg/mL)	1.4266 (±0.245)	1.324 (±0.179)	0.051
ApoB (mg/mL)	0.8028 (±0.176)	0.9015 (±0.255)	0.178
CRP (mmol/L)	**1.361 (± 1.767)**	**2.847 (± 2.101)**	**0.035**
Campesterol (mg/dL)	0.4953 (±0.279)	0.3424 (±0.194)	0.056
Sitosterol (mg/dL)	**0.4040 (± 0.196)**	**0.2716 (± 0.153)**	**0.025**
24S-OH cholesterol	64.06 (±2.540)	69.90 (±5.199)	0.296
Lathosterol (mg/dL)	0.1930 (±0.097)	0.225 (±0.130)	0.401
Campestanol (mg/dL)	3.552 (±1.385)	3.368 (±1.811)	0.729
Stigmasterol (μg/dL)	9.721 (±0.602)	11.93 (±1.115)	0.092
Sitostanol (mg/dL)	4.586 (±0.255)	4.347 (±0.436)	0.639
Lanosterol (μg/dL)	17.50 (±9.01)	21.11 (±11.17)	0.284
Dihydrolanosterol (μg/dL)	13.33 (±2.304)	14.65 (±4.385)	0.260
Desmosterol (mg/dL)	0.1401 (±0.052)	0.1295 (±0.067)	0.595
7α-OH-cholesterol (ng/mL)	72.33 (±101.0)	98.95 (±84.29)	0.382
Cholesterol (mg/dL)	185.4 (±27.51)	209.3 (±47.96)	0.071
27-OH cholesterol (ng/mL)	**185.0 (± 54.84)**	**135.0 (± 44.69)**	**0.004**
Cholestanol (mg/dL)	**0.2825 (± 0.060)**	**0.3501 (± 0.088)**	**0.010**
Brassicasterol (μg/dL)	**32.07 (± 3.802)**	**11.16 (± 1.518)**	**<0.0001**

Because TC and HDL-C levels vary by age, we also divided each cohort into two groups aged <45 years and >45 years (Table 2) with no significant differences in mean age (P = 0.088 for <45 years, P = 0.131 for >45 years). However, there are several caveats associated with our cohorts for this pilot study. First, the DS cohort has significantly more males than the control group, in both the pooled sample and after dividing each cohort into two age groups. Second, control and DS subjects were not matched for body mass index, diabetes, diet, and exercise, all of which pose significant potential confounds to our pilot results. Nevertheless, this pilot study yielded some interesting observations that could be used to design a future investigation that is sufficiently powered and adequately matched for these variables.

3.2. Serum Lipids, Lipoproteins, Sterol Precursors, and Metabolites in the Pooled Sample. No significant differences were observed between control and DS subjects with respect to TC, HDL-C, apoA-I, and apoB levels (Table 2). Consistent with this, the levels of cholesterol biosynthetic intermediates lathosterol, lanosterol, dihydrolanosterol, and desmosterol also showed no significant differences between DS and control groups. Cholesterol itself did not differ from controls either when measured by an enzymatic assay or by GC-MS (Table 2). Similarly, the levels of the rate-limiting bile acid biosynthetic marker 7α-OH-cholesterol were unchanged between DS and control groups (Table 2). Taken together, these observations suggest that global cholesterol homeostasis is not significantly altered by trisomy 21.

In humans, the cholesterol metabolite 24S-OH-cholesterol is exclusively generated in the brain by the enzyme cyp46 and plays an important role in maintaining cholesterol homeostasis in the CNS [74, 75]. 24S-OH-cholesterol easily crosses the BBB and its serum levels are therefore a marker of brain cholesterol turnover. Serum 24S-OH-cholesterol levels are not significantly different between DS and control subjects, suggesting that DS is not associated with altered cholesterol catabolism in the CNS. In contrast to 24S-OH-cholesterol, the ubiquitous oxysterol 27-OH-cholesterol is significantly reduced in DS subjects compared to controls (P = 0.004).

The inflammatory marker CRP was significantly elevated in DS subjects compared to controls (P = 0.035), as was the sterol metabolite cholestanol (P = 0.010). Although the levels of the plant stanols campestanol and sitostanol were unchanged between DS and control subjects, we observed

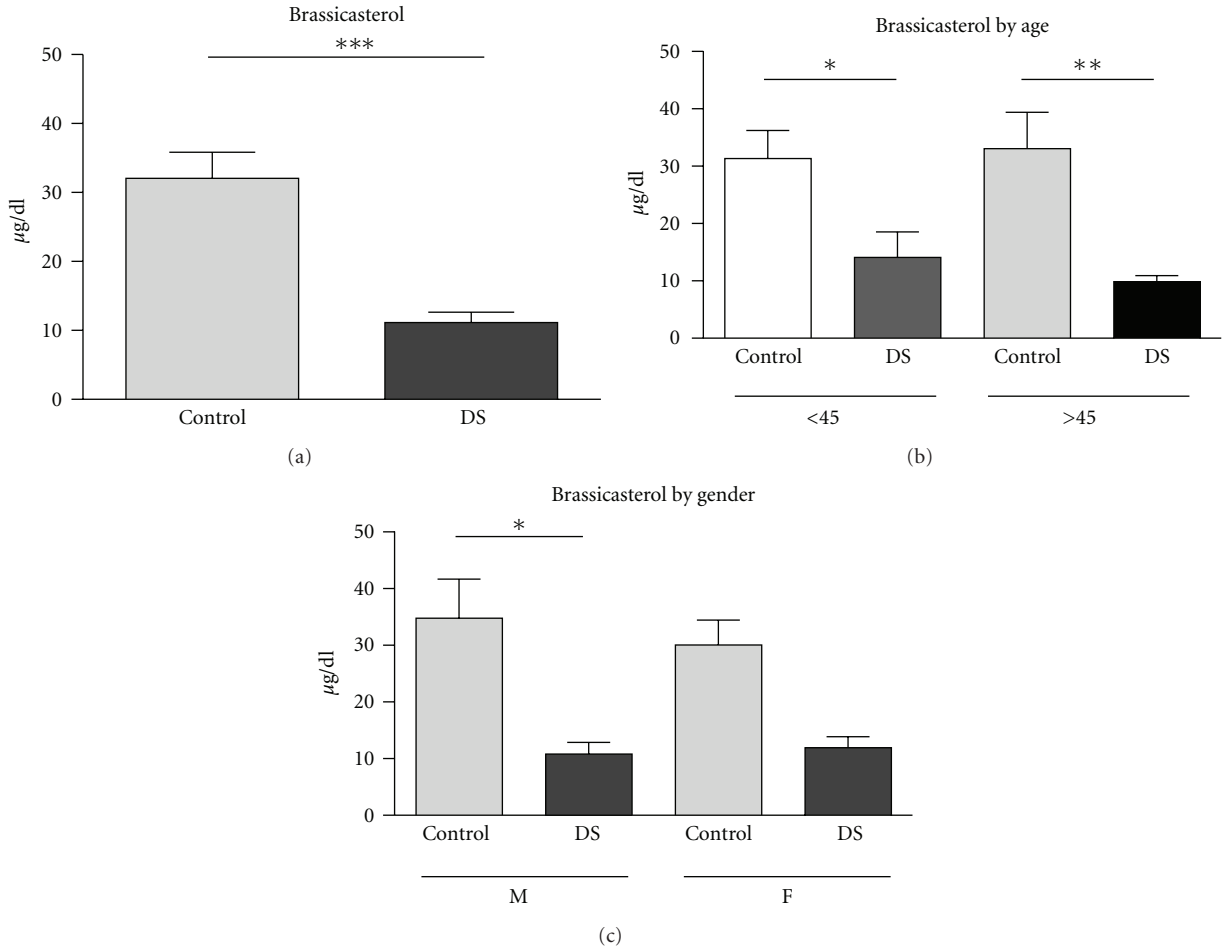

Figure 1: Serum brassicasterol levels are reduced in Down Syndrome (DS) subjects. Serum brassicasterol levels were quantified by GC-MS in $N = 20$ DS and $N = 22$ healthy controls (CON). (a) Mean and standard error of brassicasterol levels in the total CON and DS sample. (b) Mean and standard error of brassicasterol levels in the CON and DS subjects <45 and >45 years of age. (c) Mean and standard error of brassicasterol levels in male and female CON and DS subjects. Data were analysed by Students t-test. *represents $P < 0.05$, **represents $P < 0.01$, and ***represents $P < 0.001$.

that serum levels of most of the plant sterols evaluated were reduced. Most strikingly, brassicasterol levels were remarkably lower in DS subjects ($P < 0.0001$) (Table 2, Figure 1(a)). Sitosterol levels are also significantly lower in DS subjects ($P = 0.025$) and campesterol levels show a nonsignificant trend toward reduced levels in DS subjects ($P = 0.056$). Stigmasterol levels were comparable in DS and control subjects ($P = 0.092$).

3.3. Serum Lipids, Lipoproteins, Sterol Precursors, and Metabolites by Age. When divided into two groups aged <45 and >45 years, no significant differences were observed between control and DS subjects with respect to TC, HDL-C, and apoB levels (Table 3). ApoA-I levels, which were not different in the pooled sample, were significantly reduced only in DS subjects >45 years compared to controls ($P = 0.016$) (Table 3). When measured by GC-MS, cholesterol levels were significantly elevated only in DS subjects <45 years ($P = 0.008$). Similar to the pooled sample, the levels of sterol precursors lathosterol, lanosterol, dihydrocholesterol, and desmosterol did not differ when adjusted by age between DS and control subjects, nor were 7α-OH-cholesterol levels changed in either age group (Table 3). Also consistent with the pooled sample, 24S-OH-cholesterol levels remained unchanged when DS and controls subjects were stratified by age (Table 3). Serum 27-OH-cholesterol levels remained significantly lower only in DS subjects <45 years ($P = 0.010$, Table 3). After dividing by age, CRP levels significantly elevated only in subjects <45 years compared to controls ($P = 0.051$). Similarly, elevated levels of cholestanol were retained only in DS subjects <45 years ($P = 0.022$).

Consistent with the pooled sample, levels of the plant stanols campestanol and sitostanol remained unchanged when DS and control subjects were grouped by age. When stratified by age, brassicasterol levels remained greatly reduced in DS subjects <45 years ($P = 0.039$) as well as in DS subjects >45 years ($P = 0.007$) compared to age-matched controls (Table 3, Figure 1(b)). Sitosterol levels also remained significantly lower in DS subjects >45 years

Table 3: Serum analytes in control and DS cohorts by age. Analytes shown in bold are significantly different between control and DS groups by unpaired student's *t*-test. Welch's correction was applied when variances were significantly different between groups.

	Con < 45	DS < 45	*P*	Con > 45	DS > 45	*P*
TC (mmol/L)	4.859 (±0.622)	5.095 (±1.050)	0.576	4.974 (±0.793)	4.811 (±1.201)	0.727
HDL-C (mmol/L)	1.632 (±0.632)	1.357 (±0.293)	0.340	1.643 (±0.410)	1.443 (±0.268)	0.204
ApoA-I (mg/mL)	1.447 (±0.298)	1.333 (±0.179)	0.362	**1.547 (± 0.196)**	**1.317 (± 0.188)**	**0.016**
ApoB (mg/mL)	0.826 (±0.196)	0.918 (±0.203)	0.358	0.780 (±0.162)	0.891 (±0.293)	0.320
CRP (mmol/L)	0.800 (±1.872)	2.743 (±2.045)	0.051	1.233 (±1.806)	2.938 (±2.286)	0.107
Campesterol (mg/dL)	0.5406 (±0.333)	0.4378 (±0.283)	0.547	**0.4894 (± 0.219)**	**0.3015 (± 0.114)**	**0.014**
Sitosterol (mg/dL)	0.437 (±0.238)	0.352 (±0.225)	0.506	**0.422 (± 0.213)**	**0.2369 (± 0.102)**	**0.010**
24S-OH cholesterol	63.67 (±3.122)	66.50 (±15.20)	0.721	60.78 (±3.172)	71.36 (±7.038)	0.187
Lathosterol (mg/dL)	0.2039 (±0.115)	0.2703 (±0.092)	0.259	0.1951 (±0.085)	0.2054 (±0.141)	0.847
Campestanol (mg/dL)	3.639 (±1.704)	4.138 (±2.789)	0.672	3.659 (±0.990)	3.038 (±1.187)	0.207
Stigmasterol (mg/dL)	10.09 (±1.092)	13.99 (±3.103)	0.280	10.33 (±1.996)	11.04 (±3.360)	0.572
Sitostanol (mg/dL)	4.709 (±1.394)	5.338 (±3.018)	0.591	4.790 (±1.106)	3.921 (±1.183)	0.084
Lanosterol (mg/dL)	20.11 (±11.26)	21.80 (±10.60)	0.776	18.60 (±10.15)	20.81 (±11.79)	0.648
Dihydrolanosterol (mg/dL)	13.66 (±2.625)	14.25 (±4.562)	0.739	13.60 (±3.067)	14.81 (±4.473)	0.487
Desmosterol (mg/dL)	0.1522 (±0.063)	0.1413 (±0.058)	0.741	0.1352 (±0.047)	0.1244 (±0.073)	0.696
7α-OH-cholesterol (ng/mL)	86.89 (±47.13)	81.67 (±1.60)	0.919	57.78 (±34.59)	106.4 (±97.33)	0.167
Cholesterol (mg/dL)	**178.8 (± 22.16)**	**222.2 (± 32.25)**	**0.008**	192.3 (±30.57)	203.8 (±53.01)	0.564
27-OH cholesterol (ng/mL)	**219.1 (± 57.88)**	**135.0 (± 44.99)**	**0.010**	164.8 (±46.98)	135.0 (±46.27)	0.149
Cholestanol (mg/dL)	**0.2743 (± 0.070)**	**0.3727 (± 0.085)**	**0.022**	0.2976 (±0.059)	0.3404 (±0.091)	0.274
Brassicasterol (mg/dL)	**35.40 (± 17.83)**	**14.12 (± 10.90)**	**0.039**	**33.07 (± 19.02)**	**9.886 (± 3.99)**	**0.007**

($P = 0.010$) and campesterol levels were also significantly reduced in this age group of DS subjects ($P = 0.014$) compared to controls. Similar to the pooled groups, stigmasterol levels were comparable in DS and control subjects at each age examined (<45 y: $P = 0.280$, >45 y: $P = 0.572$).

3.4. Serum Brassicasterol Levels Are Significantly Reduced in Both Male and Female DS Subjects. Of all of the analytes examined, brassicasterol appears to have the most robust association with DS, being dramatically lower in both the pooled sample and in each age group. However, because a major caveat of our study is that the DS cohort had significantly more male subjects, particularly for the group >45 years, we also analysed serum brassicasterol levels independently in male and female DS subjects compared to controls. We observed significantly lower brassicasterol levels in male ($P = 0.009$) and female ($P = 0.002$) DS subjects compared to controls (Figure 1(c)).

4. Discussion

Although atherosclerosis is a multifactorial disease with environmental factors integral to its progression, several lines of evidence suggest that environmental differences alone cannot explain the apparent resistance to atherosclerosis in the DS population. The pathology reports that first described DS as an atheroma-free population compared DS subjects to other institutionalized controls [6]. Presumably, factors such as exercise, diet, and other environmental variables were comparable between these two non-community-dwelling groups, suggesting that their different atheroma burden may be independent of environmental factors [6–8]. Through dietary surveys given to a group of individuals with DS residing in the community, Braunschweig et al. concluded that people with DS consumed a comparable diet to that of the general population [30].

Being a pilot study, our results have several associated caveats. Our sample is small in size, poorly matched for gender, and lacks nutritional information. Because our goal was to compare traditional sterol lipid profiles between people with DS and healthy typically developing controls, we did not specifically recruit a group of controls with normal intellectual ability who were matched for body mass index and diet with DS subjects. This will be an essential additional control group for future studies. The lipids to be analysed in the future should be expanded to include cholesteryl esters, sphingomyelin, gangliosides, and lipids involved in signaling, as these factors can contribute to an overall change in lipidomic profiles in both AD and atherosclerosis and may offer additional insights into how such lipids may affect AD or cardiovascular risk [76, 77].

Despite these caveats, our pilot investigation suggests that the traditional atherosclerotic risk factors in people with DS generally do not differ from typically developing controls and are in accordance with several previous studies [9, 30–32]. Specifically, TC, HDL-C, and apoB were comparable in all of our subsets. Although differences in apoA-I and cholesterol measured by GC-MS did reach statistical significance in some subsets, these changes were not retained across age groups or genders. These observations, in addition to

the finding that markers of cholesterol biosynthesis and metabolism are also not significantly altered in DS subjects, suggest that the overall sterol metabolic profile in DS is similar to the general population. Our study revealed that cholestanol levels were increased in younger subjects with DS, whereas 27-OH levels were reduced, but the significance of these observations is not immediately obvious. Our study is the first to test for alterations in circulating 24S-OH-cholesterol levels in subjects with DS compared to controls. In humans, 24S-OH-cholesterol is exclusively generated in CNS neurons and easily crosses the BBB, where its levels in the circulation reflect sterol turnover in the CNS [75]. Again, no changes were observed, suggesting that overall sterol metabolism in the DS brain is similar to that in the general population.

The major conclusion of our pilot study is that serum brassicasterol levels are significantly reduced in people with DS. The mechanism responsible for this change is unclear, as there is no obvious physiological explanation for this finding. However, the reduction is robust and persists across genders and age groups. Because plant sterols are entirely derived from the diet, the lack of dietary information in the DS and controls subjects studied here is an obvious caveat to our study. However, we believe that dietary differences alone are unlikely to entirely account for the reduced brassicasterol levels in DS subjects, as community-dwelling individuals with DS have previously been shown to consume similar diets as control subjects [30] and that other diet-derived plant sterols studied here were not significantly altered between DS and control subjects. Our findings raise interesting questions about the mechanisms underlying reduced brassicaterol levels and the effects of low brassicaterol levels on atherosclerosis risk. Plant sterols have atheroprotective properties attributed to their ability to reduce cholesterol absorption, yet TC, HDL-C, apoB and most plant sterol levels were comparable between DS and control subjects. If anything, low brassicaterol levels would argue in favor of increased risk and raise questions about whether different plant sterols have distinct effects.

Although much remains to be explored in this area, it is likely that genetic factors in DS may have a larger effect compared to environmental factors in modulating atherosclerosis risk. Fetal tissues have been used to determine whether trisomy 21 leads to genetically determined differences in lipid metabolism between DS and control subjects, as the blood-placental barrier maintains separation between the fetal and maternal circulation [78]. Therefore, nearly all cholesterol present in fetal blood is synthesized by the fetus itself. One study showed that serum TC levels are elevated in fetuses with DS, and a follow-up study by the same authors demonstrated elevated cholesterol levels in DS fetal liver samples [79, 80]. Although more work needs to be done to better define the metabolic differences in DS, this area has considerable potential to establish genetically-defined baseline lipid and lipoprotein levels in people with DS. One particularly promising area of research may be to investigate the role of ABCG1 on endothelial function in DS, given that studies in preclinical models suggest that excess ABCG1 may promote endothelial resistance to triggers of atherosclerosis [20, 21]. Although there is no firm consensus, some preclinical studies support a role for ABCG1 in atherosclerosis in specific animal models [81–84]. However, none of these studies specifically investigated endothelial function in their model systems. It is conceivable that the possible protection from atherosclerosis in the DS population may not be strongly associated with plasma lipid levels but rather may be due to better endothelial function.

An important question raised by our pilot study is whether brassicaterol levels in the CSF or brain tissue of DS subjects is altered compared to controls. Decreased CNS brassicaterol levels in DS would support the previous association with validated CSF Aβ and tau biomarkers for AD in the general population [71]. An important consideration for further investigation in this area is to include young DS subjects, as our subject group is of a mean age that would already invariably exhibit AD neuropathology. It is unclear, in both the general and DS populations, if changes in brassicasterol levels precede the development of AD neuropathology or dementia. The new AD diagnostic guidelines offer an unprecedented ability to study how the trajectory of AD pathogenesis may differ in DS subjects. Our preliminary results suggest that inclusion of CNS brassicasterol measurements may also add to the possible understanding of AD pathogenesis in the unique DS population.

5. Conclusions

In people with DS, standard serum markers of sterol lipid metabolism are generally unchanged from age-matched controls and offer little insight into why DS subjects appear to have reduced prevalence of atherosclerotic disease. Further investigation into ABCG1 function, which is inherited in triplicate in trisomy 21 and plays roles in HDL metabolism and endothelial function, may prove more informative. Among the many analytes examined in our study, serum levels of the plant sterol brassicasterol levels were remarkably reduced in DS subjects relative to healthy controls across age and gender. As CSF brassicasterol levels have been reported to be reduced in AD patients and to improve the predictive power of CSF Aβ and tau levels as AD biomarkers, it will be of interest to determine whether serum and CSF brassicaterol levels are reduced in DS subjects throughout their lifespan or could be used as a prognostic biomarker of incipient AD neuropathology in DS subjects.

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Review Article

Oxidative Stress and Mitochondrial Dysfunction in Down's Syndrome: Relevance to Aging and Dementia

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Genome-wide gene deregulation and oxidative stress appear to be critical factors determining the high variability of phenotypes in Down's syndrome (DS). Even though individuals with trisomy 21 exhibit a higher survival rate compared to other aneuploidies, most of them die *in utero* or early during postnatal life. While the survivors are currently predicted to live past 60 years, they suffer higher incidence of age-related conditions including Alzheimer's disease (AD). This paper is centered on the mechanisms by which mitochondrial factors and oxidative stress may orchestrate an adaptive response directed to maintain basic cellular functions and survival in DS. In this context, the timing of therapeutic interventions should be carefully considered for the successful treatment of chronic disorders in the DS population.

1. Introduction

Down's syndrome (DS) or trisomy 21 is a prevalent genetic cause of intellectual disability due to full or partial triplication of chromosome 21 (HSA21). The presentation varies greatly between individuals. The molecular bases of this variation is "the gene dosage effect" caused by the extra chromosome 21, which leads to a global imbalance on gene expression [1]. However, the molecular mechanisms by which such gene dosage imbalance causes DS-specific abnormalities remain unclear.

Albeit trisomy 21 is the most common aneuploidy that infants can survive, the rate of miscarriage of fetuses with DS during the first trimester is almost 50% [2]. The survival rate for the first 18 years of life of DS individuals is 50.3% of the total DS population, and the greatest percent of deaths is observed during the first 5 years of life (35.9%). The death rate drops to 13.1% between 19 and 40 years, and DS individuals of 40+ years have a greater chance to live beyond 60 years of age in developed countries, especially those without congenital heart disease [3].

A remarkable feature of the syndrome is the presence of Alzheimer's disease (AD) neuropathology in the brain of nearly all DS individuals, the majority of which develop dementia with age [4]. Besides dementia, other aging features appear prematurely such as cataracts, diabetes, hair graying, leukemia, and hearing and visual impairment. Together, they define DS as a "segmental progeroid syndrome" [5–7]. Mitochondria represent both a principal source as well as a target of free radicals, which in turn cause structural damage and activate signaling pathways associated with ageing and age-related diseases [8–10]. Both oxidative stress and mitochondrial dysfunction are prominent features of DS [11–14]. The relation between oxidative stress, genome imbalances, specific HSA21 genes, and the DS phenotype has been discussed elsewhere [11, 14–17]. In this paper, we will primarily focus on mitochondrial deregulation, oxidative stress, and the emergence of an adaptive response, which may influence the timing and extent of clinical manifestations in DS.

2. Mitochondria and Oxidative Stress

Mitochondria have three major functions: generation of ATP, production of reactive oxygen species (ROS) and initiation of apoptosis. NADH and $FADH_2$ formed in glycolysis, fatty

acid oxidation, and the citric acid cycle are used to reduce oxygen to water by a series of electron carriers located in the inner mitochondrial membrane. The flow of electrons leads to the pumping of protons out of the mitochondrial matrix and the formation of a proton gradient across the inner membrane, which provides the driving force used by ATP synthase to produce ATP. This process is known as oxidative phosphorylation (OXPHOS) [18, 19]. Most of the cellular ROS are produced by electrons escaping from the electron transport chain (ETC) which are captured by O_2. Some studies suggest that as much as 2–5% of the total O_2 intake ends up forming superoxide radicals. These are scavenged by antioxidant enzymes such as mitochondrial superoxide dismutase (SOD2) and glutathione peroxidase (Gpx) [20]. Mitochondrial DNA (mtDNA) encodes 37 genes: 13 mRNAs for subunits of ETC complexes, 22 tRNAs, and 2 rRNAs operating protein translation in the mitochondrial matrix [18]. Since mtDNA is in close proximity to the ETC, it can suffer mutations under excessive ROS production, leading to impaired gene expression and further reductions in ETC efficiency. Mitochondria eventually become dysfunctional beyond repair and lose their electrochemical membrane potential (MMP). The loss of MMP activates the permeability transition pore, releasing mitochondrial material to the cytoplasm. Ultimately, this triggers the execution phase of the apoptotic process [18], which has been implicated in multiple conditions including mitochondrial diseases, DS, and age-related neurodegeneration [21, 22].

Besides mtDNA, nuclear DNA (nDNA) encodes approximately 1600 mitochondrial genes [18, 21]. Because of the split location of mitochondrial genes, mitochondrial genetics does not follow Mendelian rules. While mitochondria and mtDNA are maternally inherited [21], nuclear encoded mitochondrial genes (NEMGs) are inherited from both parents. Since each cell has thousands of mitochondria and mtDNA copies; individual differences in the ratio of normal and mutant mtDNA lead to heteroplasmy. Variations in heteroplasmy and in the energy requirements of specific cells and tissues dictate the variability in the presentation of mitochondrial diseases, not unlike what is observed in DS. The proportion of mutated mtDNAs varies spatially (depending on the cell and tissue) and temporally (over the individual's life). Thus, a particular mtDNA mutation may cause variable phenotypes [18, 23]. For example, the mtDNA mutation $tRNA^{Leu}$ A3243G has been associated with mitochondrial myopathy, encephalopathy, lactic acidosis, stroke-like episodes (MELAS), diabetes mellitus, Leigh's disease, and progressive external ophthalmoplegia (PEO) [24]. This variability in phenotypes may also be relevant to DS, where there is a high rate of mtDNA mutations and several mitochondrial genes are disproportionally expressed.

3. Mitochondria in DS and DSAD

In addition to a handful of mitochondrial genes in HSA21 whose deregulation may impair mitochondrial function, the evidence suggests that cytoplasmic inheritance of deleterious mtDNA mutations in maternal mitochondria can influence the frequency of DS in families or increase DS incidence in pregnancies from older age females [25, 26]. Mitochondrial activity is essential for spindle formation and chromosome segregation during meiosis and early embryogenesis [27]. Age appears to influence mitochondrial function in oocytes and follicular cells, and mtDNA mutations in oocytes have been found to be age related [23, 27]. Dysfunctional mitochondria have been implicated in the predisposition to chromosomal nondisjunction during the first and second meiotic divisions, in mitotic errors in embryos, and in the reduced quality and developmental potential of aged oocytes and embryos [23, 27, 28]. Thus, variable levels of mtDNA mutations in maternal mitochondria would be present in different DS individuals. Since mtDNA mutations accumulate with age, individuals starting their lives with higher mtDNA mutation rates would be more predisposed to age-related dementia. In fact, DS with Alzheimer's disease (DSAD) exhibit higher rates of mtDNA mutations in frontal cortex compared to DS and age-matched controls (Figure 1(a)).

Similar differences were observed when mtDNA mutations were analyzed in lymphoblastoid cells (LCL) (Figure 1(b)) [29], indicating a systemic increase in mtDNA mutations in DSAD. Specific mtDNA nucleotides were mutated at higher rates in DSAD and sporadic AD than in controls, and mutations in replication and transcription regulatory sequences resulting in reduced mtDNA levels and light strand gene expression were found in brains of DSAD and AD individuals [29, 30]. Consistent with these studies, a previous report indicates defective repair of mtDNA damage in DS [31]. Interestingly, DS brains without AD exhibited a slight increase in mtDNA levels, suggesting a compensatory upregulation of mitochondrial biogenesis, which disappeared in DSAD subjects. This decrease in mitochondrial biogenesis with dementia correlates with increased Aß levels and deposition, suggesting Aβ-related toxic mechanisms affecting mitochondrial biogenesis (Figure 2) [29].

4. Oxidative Stress and Mitochondrial Alterations

Increased oxidative stress in DS and AD correlates with a decrease in several mitochondrial components including complex IV nuclear encoded subunit IV, mtDNA encoded subunit I [32], complex I nuclear encoded 24 and 75 kDa subunits [33], complex V nuclear encoded β subunit and complex III nuclear encoded core protein I [23], and mitochondrial ATPase6 and mitochondrial transcription factor A (Tfam) [34]. A recent study in DS fibroblasts found a specific deficiency in complex I, increased levels of several ETC components, and increased porin levels, further suggesting that mitochondrial biogenesis is upregulated in DS. The defect in complex I was associated with decreased cAMP-dependent phosphorylation of complex I 18 kDa subunit, reduced protein kinase A activity and low basal levels of cAMP. Mitochondrial superoxide production and oxidative stress were found to be 3 times higher in DS fibroblast, which were rescued by treatment with a cAMP analog [35].

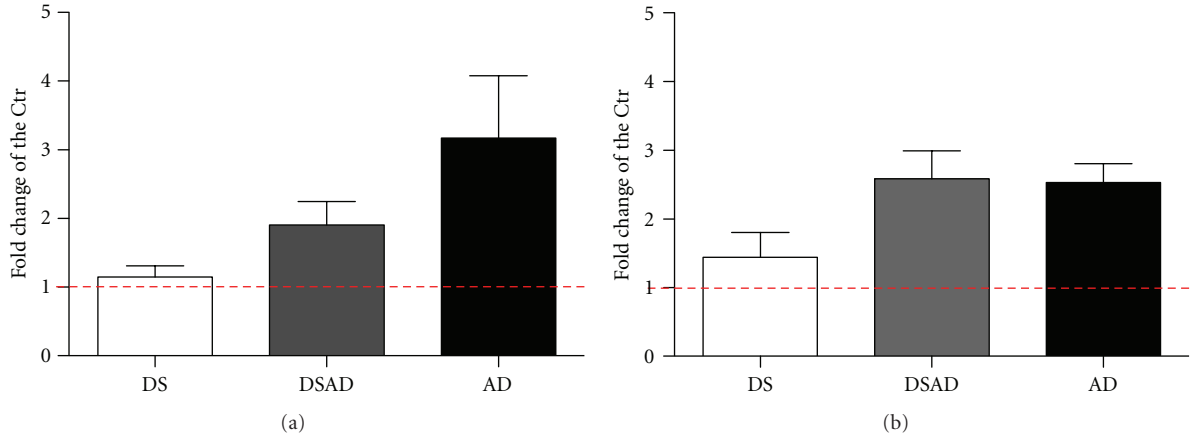

Figure 1: Accumulation of mitochondrial DNA mutations in DS, DSAD, and AD frontal cortex (a) and lymphoblastoid cell lines (LCL) (b). The graph was plotted as fold difference with respect to age-matched controls. DS brains age group: 0–40, DSAD age group: 45–68, and AD age group: 65–90. For each group 6 to 16 samples were analyzed. LCL lines for all groups (DS, DSAD, DAD, and control) were obtained from 40–60 years old donors, 6–8 samples per group. The red line shows the baseline mutation level for the control group.

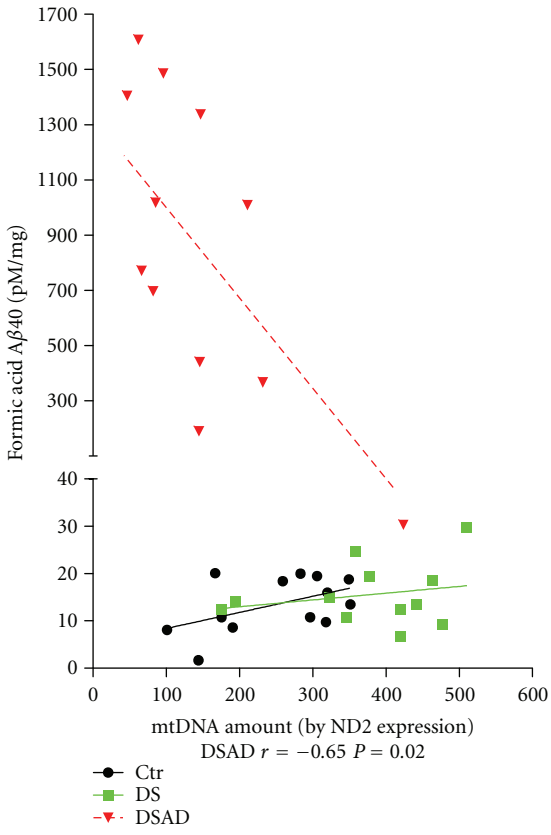

Figure 2: Levels of Aβ correlated with mitochondrial biogenesis represented as mtDNA amount. There was a significant inverse correlation between insoluble Aβ and mitochondrial DNA amount only in DSAD cases. Results reprinted from [29].

The general changes in expression of mitochondrial enzymes correlate with a downregulation of the major mitochondrial heat shock protein, HSP60 [36, 37], which is critical to prevent protein aggregation during thermal and ROS stress. In addition, a number of mitochondrial proteins are elevated in DS including mitochondrial aconitase, NADP-linked isocitrate dehydrogenase [38], and the mitochondria-targeted ES1 protein homologue [39], all of which may be part of a compensatory antioxidant response to increased mitochondrial ROS production.

5. DS and Hormesis

Based on the considerations above, it is conceivable that oxidative stress and redox changes play a dual role in DS. At low levels, they promote cellular proliferation, while at higher levels, they produce oxidative damage and initiate apoptosis [40]. Adaptive response signaling, also known as hormesis, is triggered by sublethal stress, which stimulates cellular functional changes to protect against a subsequent exposure to more severe stress [41]. Consequently, compensatory mechanisms can prepare the cell to resist higher stress levels [42].

Since mitochondria are the main source of ROS production, their role is essential in age-related oxidative damage. While abundant research supports the idea that reduced oxidative stress is associated with increased life span [43–46] several experiments showed inconsistent or even contradictory results in human studies when interventions aimed to lower ROS level [47] were unable to produce health beneficial effects [48, 49]. In a recent example, which is relevant to DS, a 2-year randomized placebo-controlled daily oral antioxidant supplementation did not improve cognitive functioning nor it stabilized cognitive decline in DSAD [50]. These findings suggest that mitochondrial ROS production could indeed trigger cellular processes that promote health and longevity. Such signaling events, or adaptive response, are observed in the context of caloric restriction (CR), one of the best intervention strategies to increase life span from yeast to mammals. In fact, CR induces mitochondrial hormesis (mitohormesis) [51] by increasing mitochondrial

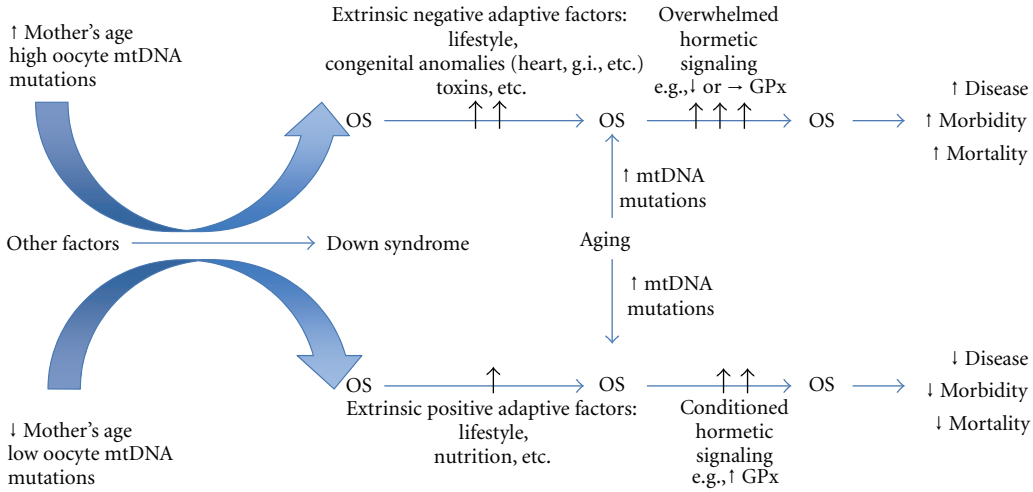

Figure 3: Modulation of DS phenotypes by oxidative stress and mitochondrial factors. Fetal oxidative stress (OS) levels could be determined by the mother's age and initial mtDNA mutation levels in oocytes. Besides the genetic/intrinsic factors that create the genomic instability in DS, environmental factors and lifestyle modulate the initial OS further. Since all these factors that play a role in the level of OS differ individually, the OS-related changes will also be observed in variation. Simply, while the low level of OS could initiate the positive adaptive response by activating proper defense signaling, high level of OS will start destructive signaling where the adaptive response could not be able to accommodate the clearance of the damage. More positive factors (e.g., lifestyle, advantageous genetic background—mitochondrial haplotype, APOE, BDNF genotype, etc.—and nutrition) will feed the adaptive response positively, while negative factors (e.g., congenital defects, sedentary lifestyle, genotypes, etc.) will increase the OS further. In both low and high levels of initial OS conditions, aging will affect this process negatively by increasing OS, such as increasing mtDNA mutation accumulation and decline in mitochondrial functions. Under increasing OS conditions with aging, individuals with DS will be prone to develop more morbid conditions and prone to death depending on their initial adaptive response signaling. In other words, negative factors will lead to earlier clinical manifestations of age-related conditions, while positive adaptations (e.g., conditioned hormetic signaling) may support normal cellular and systemic functions for longer periods of time.

respiration and elevating mitochondrial ROS production without changing ATP production [52].

A prominent sensor related to hormesis is the Keap1-Nrf2-ARE signaling complex. Under normal redox conditions, the transcription factor NFE2-related factor 2 (Nrf2) binds to Kelch-like ECH-associated protein 1 (Keap1) in the cytosol leading to its proteasomal degradation [53]. Keap1 is a cysteine-rich protein that senses redox changes in the cell. Under oxidative stress, conformational changes in Keap1 lead to its dissociation from the Nrf2-Keap1 complex and to the translocation of free Nrf2 into the nucleus, where it binds to antioxidant response element (ARE) regions in the genome, and activates the expression of stress response genes [54, 55]. So far, there is no complete information on Nrf2/Keap1 genes, protein levels or activities in DS. However, a recent study comparing gene expression profiles in DS and euploid astrocytes found that Nrf-2-associated oxidative stress response genes were differentially regulated in DS, supporting the presence of hormesis in DS [56]. Additional evidence of hormesis in DS comes from experiments showing increased activity of mitogen-activated protein kinases (MAPKs), including ERK1/2, SAPKs, and p38 in DS and AD brains [57]. MAPKs phosphorylate Nrf2 enabling its dissociation from the Nrf2/Keap1 complex [58]. *GPx* and *catalase* are also Nrf2 target genes carrying ARE sequences in their promoters [59]. Interestingly, higher intellectual function in DS correlated with increased expression of GPx, which could be part of the adaptive response in those individuals [60].

Nrf2 also interacts with PPARγ, PGC1α, and PI3K/Akt, all of which participate in mitochondrial biogenesis [61]. Thus, these factors may underlie mtDNA increase [29] and mitochondrial biogenesis [35] in DS cells. Finally, a generalized downregulation of mitochondrial activity has been observed in different DS cell types including neurons, astrocytes, pancreatic β cells, endothelial cells, and fibroblasts, which is consistent with a cellular adaptation to reduce ROS production and prevent cellular injury [56]. However, additional stressors and/or challenges in the form of infections, seizures, age-related loss of function, and so forth can eventually exhaust the capacity of the functional adaptations to avert cellular damage. In this context, chronic respiratory infections and multiple signs of early senescence such as cataracts, skin atrophy, seizures, leukemia, and AD-type neuropathology may be the result of oxidative stress, mitochondrial dysfunction, and additional factors acting systemically or in specific organs and tissues. Thus, the severity of the DS phenotype may be the result of the initial level of mitochondrial mutations, the accumulation of oxidative damage, and the magnitude of the cellular adaptations triggered by these changes. Activation of an early adaptive response by initial sublethal levels of stress may translate in a longer survival. However, the combination of chronic stress and age-related changes would result in

the premature and accelerated development of age-related conditions such as dementia and AD pathology.

One consequence of the considerations above is that not only the compounds but also the timing of treatment options should be carefully considered in DS patients. For example, long-term treatments designed to reduce oxidative stress may not add any incremental benefit on top of the changes driven by hormesis. Interventions would be more effective if introduced at the very onset of stress or disease. In fact, recent findings indicate that exercise-induced oxidative stress ameliorates insulin resistance and generates an adaptive response enhancing the endogenous antioxidant defense capacity [62]. However, supplementation with antioxidants may preclude the health-promoting effects of exercise in humans [62]. Thus, under normal conditions, antioxidants may not help and may even interfere with hormesis. According to this hypothesis, they would be most effective when an additional stressor is present.

In conclusion, DS is the result of a whole genome imbalance caused by triplication of HSA21 genes. The severity and spectrum of the syndrome vary greatly. Besides oxidative damage, mtDNA mutations and mitochondrial dysfunction emerge as important modulators of DS phenotypes. This variability is further influenced by an adaptive cellular response to stress. A comprehensive and detailed analysis of signature pathways unique to hormesis will be required to fully assess the role of the adaptive response in DS (Figure 3). Key elements of hormesis may be valuable predictors of disease onset and treatment outcomes in DS individuals.

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Research Article

Ophthalmic Disorders in Adults with Down Syndrome

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A myriad of ophthalmic disorders is associated with the phenotype of Down syndrome including strabismus, cataracts, and refractive errors potentially resulting in significant visual impairment. Ophthalmic sequelae have been extensively studied in children and adolescents with Down syndrome but less often in older adults. In-depth review of medical records of older adults with Down syndrome indicated that ophthalmic disorders were common. Cataracts were the most frequent ophthalmic disorder reported, followed by refractive errors, strabismus, and presbyopia. Severity of intellectual disability was unrelated to the presence of ophthalmic disorders. Also, ophthalmic disorders were associated with lower vision-dependent functional and cognitive abilities, although not to the extent that was expected. The high prevalence of ophthalmic disorders highlights the need for periodic evaluations and individualized treatment plans for adults with Down syndrome, in general, but especially when concerns are identified.

1. Ophthalmic Disorders in Adults with Down Syndrome

Down syndrome is the most prevalent genetic disorder associated with intellectual disability and is due to the presence of complete or partial triplication of chromosome 21 [1]. It is associated with a characteristic physical and cognitive phenotype, although almost every aspect of the phenotype shows variability in terms of occurrence and severity [2, 3]. Down syndrome carries with it an increased risk of congenital heart defects, hearing loss, autoimmune diseases, shortened life expectancy, early onset Alzheimer's disease, and other concerns related to health and aging that also include multiple ophthalmic disorders [4–7]. Earlier studies have indicated increased risk for abnormality in virtually all structures of the eye including the lid, iris, cornea, lens, and retina [8–11]. As a consequence, *nystagmus*, *strabismus*, *keratoconus*, *amblyopia*, *cataracts*, and *refractive errors* are prevalent in this population potentially resulting in significant visual impairment [12, 13] (see Appendix for brief definitions of italicized terms). While no specific ophthalmic disorder seems to be pathognomonic of Down syndrome, many individuals present with a combination of conditions [12, 14].

The ophthalmic sequelae in children and adolescents with Down syndrome have received considerable attention [12, 15–18], but the prevalence of vision problems in older adults has been reported less often. The life expectancy of adults with Down syndrome has increased dramatically over the last several decades [19, 20] and as a consequence, they are prone to experience health problems associated with advancing age, such as visual functioning deficits that are likely to be similar to or more severe than those seen in adults without intellectual disability. The studies that do exist on vision in adults with Down syndrome have generally found that the number and severity of ophthalmic disorders

increase with age [9, 21–27]. Van Schrojenstein Lantman-de Valk et al. [23] examined the sensory functioning of older individuals with intellectual disability in the Netherlands, who were between 50 and 59 years of age and found that visual impairment occurred in 46% of adults with Down syndrome. This number increased significantly with age such that 85% of people with Down syndrome, 60 years of age and older, experienced visual impairment [9, 21, 27]. The age-specific prevalence for specific ophthalmic disorders has rarely been reported [28] although van Schrojenstein Lantman de Valk et al. [23] found that the prevalence of cataracts in adults with Down syndrome increased from 16% of individuals between 50 and 59 years of age to 63% of individuals 60 years of age and older (also see [29]). Van Buggenhout et al. [27] found that the severity of ophthalmic disorders increased with age in adults with Down syndrome. While moderate-to-severe vision loss was reported in 18% of individuals between 30 and 39 years of age, prevalence increased to 28% for individuals between 40 and 49 years of age and to almost 50% for individuals between 50 and 59 years of age. Thus, it is likely that changes in vision are among the features of atypical aging seen in individuals with Down syndrome in middle age.

Prevalence of ophthalmic disorders has been found to increase dramatically with severity of intellectual impairment in individuals with Down syndrome ([9, 30]; cf. [15]). For example, Evenhuis et al. [9] observed visual impairment in 4.5% of individuals with mild or moderate intellectual disability but in 74% of individuals with severe or profound intellectual disability. Several researchers examined the relation between severity of intellectual disability and prevalence of specific disorders [10, 31, 32]. McCulloch et al. [31] found that 25% of individuals with mild intellectual disability had strabismus compared to 60% of individuals with profound intellectual disability. Further, *esotropia* (the form of strabismus where one or both eyes tend to drift inward) was typically found in those with milder disabilities, whereas *exotropia* (where one or both eyes tend to drift outward) was most common in those with more severe disabilities [31]. Other associations with severity of intellectual impairment have been found for *visual acuity* as well as refractive errors [31, 32].

Intellectual disability results in significantly impaired functioning, but when it cooccurs with visual impairment, overall disability can be exacerbated and quality of life may be reduced. Visual impairment has been found to significantly decrease independent living skills, communication and language skills, social skills, and initiative and persistence [33, 34]. The aim of the present study was to evaluate the characteristics and prevalence of specific ophthalmic disorders in older adults with Down syndrome (from 30 to 83 year olds) and to determine if the presence of ophthalmic disorders affects adaptive behavior and cognitive status. In addition, inclusion of individuals with a wide range of intellectual disability (FSIQ range = 20–71) enabled the examination of how prevalence of ophthalmic disorders varies as a function of intellectual disability.

Table 1: Participant characteristics.

Characteristic	Down syndrome ($n = 455$)	
Age (Mean, SD)	(50.93, *7.85*)	
Computed FSIQ[1] (Mean, SD)	(32.49, *9.37*)	
	n	%
Age group		
30–39	23	5.1
40–49	188	41.3
50–59	184	40.4
60–69	50	11.0
70–79	9	2.0
80+	1	.2
Level of intellectual disability		
Mild	30	6.9
Moderate	167	38.3
Severe	114	26.1
Profound	125	28.7
Sex		
Female	316	69.5
Male	139	30.5
Presence of ophthalmic disorders	353	77.6

[1]IQs were unavailable for 19 adults (4.2%).

2. Method

2.1. Human Subject Approvals. This study was approved by the Institutional Review Boards of the New York State Institute for Basic Research in Developmental Disabilities and the Johns Hopkins University School of Medicine. Participants gave their assent for all procedures, and for each participant, an authorized representative provided informed consent.

2.2. Participants. The participants were 455 adults with Down syndrome, who were enrolled in a larger multidisciplinary study focused on aging and dementia (see [35, 36] for inclusion criteria). Table 1 presents the demographic characteristics of the participants. There was a preponderance of females (69.5%), which reflects the interests and sampling procedures of our overall program, one goal of which was to investigate women's health issues and aging. Multiple IQs were obtained from clinical records and testing typically occurred when the participants were children or young adults. The specific IQ tests and dates of administration were also recorded. We generated a "consensus Full Scale/Composite IQ" for each participant using either the results actually obtained or, in cases where data were only available from the Wechsler Adult Intelligence Scale [37], an estimated "Stanford-Binet-equivalent" was calculated to address the compelling evidence that the various editions of the Wechsler Adult Intelligence Scale generate substantially higher IQs for this population compared to other assessments [38].

Down syndrome was confirmed cytogenetically for 368 (82.9%) individuals; 328 (89.1%) had full trisomy 21, 25 (6.8%) had trisomy 21 mosaicism, and 15 (4.1%) had an

autosomal translocation. The families of 76 (16.7%) individuals refused consent for a blood sample, and we were unable to obtain a blood sample from another 12 individuals (2.6%). These 88 individuals were confirmed to have trisomy 21 based on phenotype.

2.3. Materials and Procedures. Participants were comprehensively evaluated at approximately 18-month intervals with an assessment battery that included detailed review of medical records, informant interviews, direct assessment of a variety of cognitive functions, collection of blood samples, and, for a selected subsample, a neurological examination. The primary data for this study came from the medical records of participants obtained from clinical or agency files and examined upon their entry into the study. These records were hand-searched and data regarding all diagnoses and clinically significant health problems were extracted and entered onto a standardized form following a protocol developed in conjunction with the broader research program. The form included questions pertaining to all body systems. It also included the date and course of treatment for specific conditions and demographic information. The presence or absence of specific ophthalmic disorders was examined for this report.

As part of our longitudinal study, we examined the cognitive abilities and behavioral functioning of all study participants. For the current study, we report on measures where performance should be especially sensitive to visual processing and, for comparison, those that should be relatively independent of visual processing. The medical chart review, cognitive, and adaptive measures were collected contemporaneously. The American Association on Mental Retardation (AAMR)—Adaptive Behavior Scale (ABS-Part One) [39, 40], an informant-based assessment measuring a variety of functional domains, was used to examine adaptive competence and functional abilities. The skills examined within Part One are grouped into 10 behavior domains reflecting independent functioning, physical development, economic activity, language development, numbers and time, domestic activity, vocational activity, self-direction, responsibility, and socialization. The 10 adaptive domain scores were summed to create an overall index of adaptive functioning with a maximum possible score of 280.

The cognitive abilities of participants were evaluated with direct testing. Measures sensitive to visual processing included the Block Design subtest of the WISC-R [41] plus a series of simpler items referred to as the Extended Block Design test [42]. Both tasks involved reproducing visual patterns from models with red and white Kohs blocks. These tests provided a measure of visuospatial organization, with performance requiring both an analysis of visual details and the synthesis of the final design. Procedures were consistent with those described in the WISC-R manual with the exception that testing always began with the simplest design, a single block, and progressed in difficulty to 2×2- and 3×3-block designs. Each trial had a time limit, and the score represents the number of designs completed successfully within that time frame. The dependent measure was the sum of the raw scores on these two tests (scaled scores were unavailable for the ages of our participants), with a maximum possible score of 78.

The Beery-Buktenica Developmental Test of Visual-Motor Integration was used to ascertain construction ability [43]. The task requires participants to copy simple figures using paper and pencil, starting with one straight line (in both a horizontal and vertical orientation) and a circle. Figures progressively increase in complexity by the addition of lines and shapes. A single summary score was generated to reflect overall performance using standard scoring procedures with a maximum possible score of 27.

An adaptation of the McCarthy [44] Verbal Fluency Test was one of the "nonvisual" tasks included in our battery. It requires participants to name as many foods, animals, or clothes (two of these categories are administered in any given test cycle) as fast as possible within 20 s. A summary score was generated by adding the number of correct responses for the two categories.

Another test independent of visual processing was a modified version of the Selective Reminding Test [45, 46]. Eight items from a single semantic category (animals or foods) are presented verbally followed by 6 trials of free recall. After the first trial, only those items that were not recalled on an immediately preceding trial are represented for learning on the next trial. The Selective Reminding Test generates multiple scores that reflect the efficiency of various memory processes [45, 47], but our primary measure of interest was the total number of items recalled over the 6 trials with a maximum score of 48.

SYSTAT 12 was used for all analyses. Chi-square analyses were conducted on categorical data. Graphic analyses were conducted on these data to determine overall significance for the set of dependent variables following procedures similar to those described by Schweder and Spjøtvoll [48]. This strategy avoids the substantial loss of power associated with a straightforward Bonferroni correction for multiple tests yet addresses concerns associated with potential inflation of type-I error probability. The General Linear Model module was used for analyses of continuous data.

3. Results

It was exceedingly common for older adults with Down syndrome to have an ophthalmic disorder. The medical records of 77.6% (353 of 455) adults with Down syndrome indicated they had at least one ophthalmic disorder. We found an association between age and the prevalence of having at least one ophthalmic disorder such that, as a group, individuals having an ophthalmic disorder were 2.5 years older than those who did not, $F(1,454) = 8.35$, $P = .004$. The association between sex and the prevalence of having at least one ophthalmic disorder was not significant, $\chi^2(1, N = 455) < 1$.

Data regarding the prevalence of specific ophthalmic disorders are summarized in Table 2. A wide variety of ophthalmic disorders was noted in participants' medical charts.

Table 2: Common ophthalmic findings and percentage prevalence.

Ophthalmic conditions	
Amblyopia	13 (2.9%)
Aphakia	13 (2.9%)
Blepharitis	46 (10.1%)
Legal blindness	35 (7.7%)
Cataracts	191 (42.0%)
Conjunctivitis	61 (13.4%)
Diabetic retinopathy	0
Dry eye	4 (.9%)
Glaucoma	9 (2.0%)
Keratoconus	13(2.9%)
Macular degeneration	8 (1.8%)
Nystagmus	16 (3.5%)
Presbyopia/*hyperopia*	57 (12.5%)
Pseudoaphakia	11 (2.4%)
Pterygium	10 (2.2%)
Ptosis	3 (.7%)
Refractive error	115 (25.3%)
Astigmatism	52 (11.4%)
Myopia	88 (19.3%)
Retinal detachment	2 (.4%)
Retinitis pigmentosa	1 (.2%)
Strabismus	96 (21.1%)
Esotropia	79 (17.4%)
Exotropia	2 (.4%)

Cataracts were the most frequent ophthalmic disorder reported for adults with Down syndrome, affecting 191 of 455 (42%) individuals. Refractive errors were the second most frequent disorder, reported for 115 adults (25%), with *astigmatism* and *myopia* as the leading causes. Strabismus was reported in 21.1% and *presbyopia* in 12.5% of adults with Down syndrome. Legal blindness was reported in 7.7% of adults with Down syndrome. Keratoconus and nystagmus have been reported in previous studies as conditions frequently causing visual impairment in individuals with Down syndrome but were only noted in 2.9% and 3.5% of individuals in our study, respectively. *Blepharitis* and *conjunctivitis*, two inflammatory conditions of the eye that are unrelated to visual impairment, were reported for 10.1% and 13.4% of our sample, respectively. All other eye conditions were reported in small numbers.

Several disorders showed an association with age. Cataracts were more common for the older individuals, F (1,453) = 24.83, $P < .001$, while astigmatism, F (1,453) = 13.16, $P < .001$ and refractive errors, F (1,453) = 12.05, $P < .001$ were more frequently reported for younger individuals. The presence of all other ophthalmic disorders were found to be unrelated to age.

3.1. The Prevalence of Ophthalmic Disorders and the Severity of Intellectual Disability. Overall, the prevalence of having at least one ophthalmic disorder was not significantly different among intellectual disability severity groups for adults with Down syndrome. With one exception, this was also our finding for specific ophthalmic conditions. Individuals who were legally blind were more likely to have profound intellectual impairment (24 out of 33 legally blind participants) compared to their peers who were not legally blind, χ^2 (3, $N = 436$) = 34.11, $P < .001$.

Table 3: Age-related prevalence of cataracts.

Age (years)	Down syndrome (%)	General population in United States without intellectual disability[1]
30–39	13.0%	—[2]
40–49	37.8%	2.5%
50–59	42.9%	6.8%
60–69	60.0%	20.0%
70–79	77.8%	42.8%
80+	100.0%[3]	68.3%

[1]The Eye Diseases Prevalence Research Group (2004a) [49] and summary data available at: http://nei.nih.gov/eyedata/pbd_tables.asp.
[2]Data unavailable.
[3]Only one participant in this age category.

3.2. Cataracts. Because prevalence of cataracts was high, we examined cumulative incidence by age and treatment plans for individuals with this condition. Two individuals had congenital cataracts. Congenital cataracts are considered to be a distinct phenomenon, and the two affected individuals were, therefore, excluded from these analyses.

The average age in which an individual with Down syndrome was diagnosed with cataracts was 48.43 years (SD = 9.87). Prevalence of cataracts was unrelated to intellectual disability severity, but was related to age, as discussed previously. Table 3 presents summary prevalence data for each 10-year age interval for individuals with Down syndrome and the US national estimates for the general population (see [49]). Clearly, prevalence is higher for adults with Down syndrome, who are in their 40 s through 60 s, χ^2 (2, $N = 455$) = 1246, $P < .10^{-6}$.

A reconstructed cohort design [50] was used to estimate cumulative incidence of cataracts, in which each participant was considered to be at risk from birth until their current age (if unaffected) or until the age at which they received a diagnosis. A Kaplan-Meier Survival Analysis was used to estimate time-to-diagnosis. Figure 1 clearly shows that risk for individuals with Down syndrome increased with age quite rapidly beginning at approximately 40 years of age.

3.2.1. Treatment Plans. A number of treatment options were prescribed, typically dependent on the resulting degree of vision loss experienced by an individual. The most frequent treatments included: (a) surgery with intraocular lens implantation, (b) an increase in prescription strength of glasses, or (c) surveillance for increasing vision loss.

For almost half of the individuals with diagnosed cataracts, no treatment was undertaken at the time of diagnosis (45.3%). Typically a comment was noted in the medical

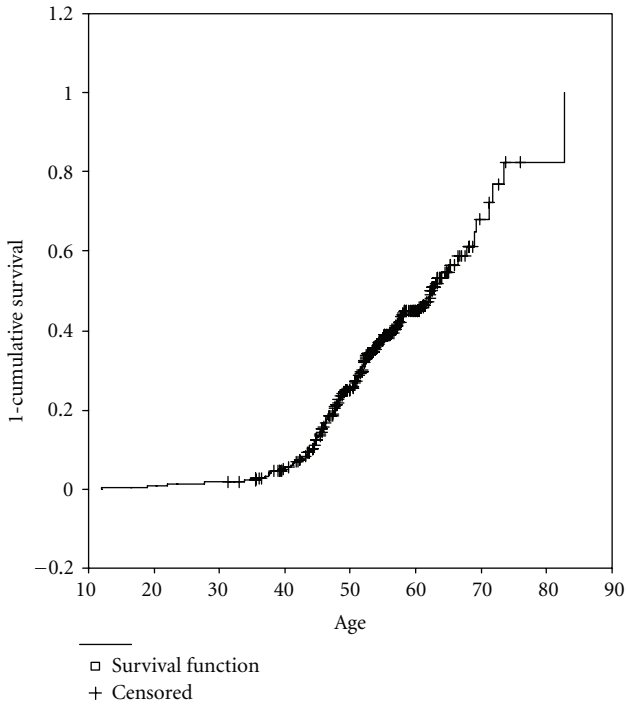

Figure 1: A Kaplan-Meier survival analysis stratified by age of cumulative incidence of cataracts for participants with Down syndrome.

record that the condition was in the early stages and was not sufficiently advanced to warrant surgery, along with a recommendation for reexamination to evaluate disease progression. Cataract surgery was reported for a relatively small number of individuals with the condition, 15.6%. For 11% of adults with Down syndrome, a change in eyeglass prescription was ordered at the time of diagnosis. The medical charts of 22.9% of individuals did not specify any treatment at the time of diagnosis.

3.3. Effects of Ophthalmic Disorders on Cognitive and Adaptive/Behavioral Function. To determine the impact of ophthalmic disorders on adaptive behavior and cognition, individuals with and without ophthalmic disorder(s) were compared on a number of performance measures dependent on visual processing (AAMR Adaptive Behavior Scale, the Block Design Test, and the Beery-Buktenica Developmental Test of Visual-Motor Integration) and those that were independent of visual processing (the Verbal Fluency Test and the Selective Reminding Test). Table 4 presents the means for these measures as a function of ophthalmic status. We excluded cases where blepharitis, conjunctivitis, and/or dry eye were the only conditions reported, reasoning that they do not usually cause impairment in visual functioning. An analysis of covariance was conducted where ophthalmic status (with and without an ophthalmic disorder(s)) was the between-subjects measure and IQ was the covariate. (The number of participants that completed each test differed between tests and therefore degrees of freedom varied as well.) In adults with Down syndrome, scores on measures that relied on visual processing were related to overall ophthalmic status, although the effect sizes were small (AAMR Adaptive Behavior Scale, F (1,425) = 3.95, P = .048, Cohen's d' = .193; the Block Design Test, F (1,322) = 7.84, P = .005, Cohen's d' = .312; the Beery-Buktenica Developmental Test of Visual-Motor Integration, F (1,338) = 3.95, P = .048, Cohen's d' = .216). We next examined the effects of specific ophthalmic disorders expected to have the most substantial effects on quality of life. Being legally blind had a detrimental effect on adaptive behavior, visuospatial organization, and construction ability although the effect sizes were small (AAMR Adaptive Behavior Scale, F (1,425) = 4.28, P = .039, Cohen's d' = .201; the Block Design Test, F (1,322) = 4.23, P = .041, Cohen's d' = .229; the Beery-Buktenica Developmental Test of Visual-Motor Integration, F (1,338) = 4.10, P = .044, Cohen's d' = .220). Having cataracts also had a detrimental effect on performance (AAMR Adaptive Behavior Scale, F (1,425) = 20.44, P < .001, Cohen's d' = .439; the Block Design Test, F (1,322) = 20.78, P < .001, Cohen's d' = .508; the Beery-Buktenica Developmental Test of Visual-Motor Integration, F (1,338) = 12.55, P < .001, Cohen's d' = .385). Individuals with presbyopia, astigmatism, myopia, or strabismus performed comparably on all measures compared to individuals that did not have these conditions. For tasks that were independent of visual processing, the performance of individuals with and without an ophthalmic disorder and with or without any of the above specific ophthalmic conditions was comparable.

Table 4: Adjusted least square means and standard errors for adaptive behavior and cognitive measures as a function of ophthalmic status.

Performance measure	With ophthalmic disorders	Without ophthalmic disorders
Functions dependent on visual processing		
AAMR-Adaptive Behavior Scale	169.12 (2.73)	179.81 (4.63)
Block Design Test	10.09 (.50)	12.71 (.79)
Beery-Buktenica Developmental Test of Visual-Motor Integration	8.28 (.24)	9.19 (.39)
Functions independent on visual processing		
Selective Reminding Test	23.11 (.82)	24.03 (1.27)
Verbal Fluency Test	5.66 (.24)	5.68 (.24)

4. Discussion

The examination of medical records has shown that adults with Down syndrome are at an increased risk for ophthalmic disorders with advancing age. The chances of having at least one ophthalmic disorder increased significantly with age and older participants had a greater number of these disorders than younger participants. It was also clear that in adults with Down syndrome, specific ophthalmic disorders are closely related to the age of the individual. We found that while

astigmatism and refractive errors were more prevalent in younger individuals, cataracts and blepharitis were more common in older individuals.

Contrary to previous studies, the prevalence of ophthalmic disorders was unrelated to severity of intellectual disability, with the one exception being that individuals who were legally blind were more likely to have profound intellectual disability. Given that visual processing is a relative strength for individuals with Down syndrome, this finding may reflect atypically severe consequences of visual impairment on cognitive development, but at this point it seems clear that valid interpretation will be dependent upon further investigation.

Cataracts were the most prevalent ophthalmic disorder recorded in medical charts for participants. As expected, prevalence increased with advancing age, and our data indicates that individuals with Down syndrome were significantly younger than individuals in the general population at the time of diagnosis [49]. This was consistent with an extensive body of literature documenting that people with Down syndrome show some signs of accelerated biological aging (e.g., [3, 51–53]). At the time of initial diagnosis, generally no treatment was prescribed for adults with Down syndrome and cataract surgery was reported infrequently. Many of the medical charts included a note that the condition was mild at the time of diagnosis and did not require treatment. We could not find comparable data on treatment prescribed at the time of diagnosis for adults in the general population, but further monitoring without immediate treatment is an accepted option within standard clinical practice.

As found in other studies, blepharitis and conjunctivitis, both inflammatory conditions of the eye, were found to be common conditions in individuals with Down syndrome. Blepharitis may be related to the narrow, slanted palpebral fissures characteristic in individuals with Down syndrome [54] or an increased susceptibility to infection associated with the impact of trisomy 21 on the immune system [22, 55, 56].

Severe visual impairment in adults without intellectual disability is known to negatively interfere with the ability to perform activities of daily living, especially those that rely on vision [57]. For example, difficulty with mobility [58, 59] and sleep problems [60] have been reported for older adults with low vision or blindness. Concerns about general safety may also come into play [53]. In individuals with intellectual disability, Evenhuis et al. [33] concluded that visual impairment compounds preexisting disability. We observed that in individuals with Down syndrome ophthalmic disorder(s) negatively affected adaptive behavior and cognitive functions that rely on visual processing. This was in contrast to the finding that individuals with and without ophthalmic disorder(s) performed comparably on selected skills that were independent of visual functioning (e.g., episodic memory and verbal fluency). We also observed that not all ophthalmic disorders were equally detrimental to adaptive behavior or cognition. Being legally blind had the most serious impact on participants' adaptive behavior skills and cognitive functioning, as one would expect, and having cataracts proved also to be detrimental. However, individuals with ophthalmic disorders were not affected to the extent that we expected. It is possible that ophthalmic disorders are being detected and treated appropriately in this population to a greater extent than previously supposed (cf. [61]), at least within networks serving our study participants.

An important limitation of the present analysis is the reliance on data from medical charts. Medical charts can be inaccurate or incomplete compared to direct examination. For example, charts frequently made no mention of treatments prescribed for ophthalmic conditions, but that could be either because no treatments were provided or no notation of provided treatments were made.

Our results have important implications with respect to the ophthalmic care of adults with Down syndrome. The high prevalence of ophthalmic disorders highlights the need for periodic evaluations of adults with Down syndrome to identify age-related changes and other pathological eye conditions. In an IASSID International Consensus Statement, Evenhuis and Nagtzaam [62] proposed that planned vision screening and examinations for adults with Down syndrome should begin by age 30 and be conducted at least every five years. Pueschel et al. [63] and Van Buggenhout et al. [27] alternatively recommend more frequent assessments, at least every 2 years in adult patients with Down syndrome and increasing in frequency with advancing age. The present findings confirm the need for regular eye examinations, and the possibility of impaired vision needs to be investigated whenever declines in functional abilities occur in an older adult with Down syndrome.

Appendix

Definitions of Ophthalmic Terms

Amblyopia (Lazy Eye): Poor vision in one or both eyes that is not associated with any specific pathology and that persists after the correction of refractive errors.

Aphakia: Absence of the lens of the eye due either to surgical removal, a perforating wound or ulcer, or a congenital abnormality.

Astigmatism: Unequal curvatures along the different meridians in one or more of the refractive surfaces of the eye.

Blepharitis: Chronic inflammation of the eyelids.

Cataract: A clouding of the crystalline lens of the eye varying from a mild to complete opacity and resulting in the obstruction of the passage of light.

Conjunctivitis: Acute inflammation of the conjunctiva, the outermost layer of the eye and the inner surface of the eyelids. It is most commonly caused by an allergic reaction or an infection.

Cornea: The clear front window of the eyeball.

Diabetic Retinopathy: A condition, which causes progressive damage to the blood vessels of the retina resulting from complications of diabetes mellitus.

Dry Eye Syndrome (Keratitis Sicca): Chronic lack of lubrication and moisture in the eye.

Esotropia: A form of strabismus in which one or both eyes turns inward.

Exotropia: A form of strabismus in which one or both eyes are deviated outward. It is the opposite of esotropia.

Glaucoma: A group of diseases that damage the optic nerve and results in progressive and irreversible vision loss and blindness. It is frequently, although not always, associated with increased fluid pressure of the eye.

Hyperopia (Farsightedness): A refractive defect of the eye whereby near objects appear blurred because the image is focused in back of the retina rather than directly on it.

Keratoconus: A degenerative noninflammatory disorder of the eye in which structural changes within the corneal curve cause it to thin and subsequently to deform the shape of the cornea to a more conical shape from its normal gradual curve.

Myopia (Nearsightedness): A refractive defect of the eye whereby distant objects appear blurred because an image is focused in front of the retina, in the vitreous, rather than on it.

Nystagmus: Refers to the rhythmic, repetitive, oscillating, involuntary eye movements that occur when a large portion of the visual field moves constantly in a horizontal direction and can contribute to decreased vision. The movements consist of a slow phase in which the moving field is tracked (smooth pursuit), followed by a rapid "return" movement (saccade); this pattern is repeated until the field stops moving. In pathological cases, nystagmus can occur in the absence of a moving stimulus.

Optic Neuritis: Is an inflammation of the optic nerve that may result in a complete or partial loss of vision.

Presbyopia: Is the progressively diminishing ability to focus on nearby objects resulting from the loss of elasticity of the crystalline lens that occurs with advancing age.

Pseudoaphakia: A congenital condition in which the crystalline lens has degenerated and been replaced by mesodermal tissue.

Pterygium: Refers to a triangular thickening of the conjunctiva (outer coating of the eye) that grows onto the cornea causing redness, irritation, and tearing. If it grows large enough, it may interfere with vision.

Ptosis: Drooping of the upper eyelid in one or both eyes caused when the muscles that raise the eyelid (levator and Müller's muscle's) are not strong enough to do so properly.

Refractive Errors: Errors in the focusing of light, for example, myopia.

Retinal Detachment: A disorder of the eye in which the inner layers of the retina separate from the underlying layer of supportive tissue, the retinal pigment epithelium.

Retinitis Pigmentosa: A group of inheritable degenerative retinal diseases in which abnormalities of the photoreceptors (the rods and cones) or the retinal pigment epithelium led to progressive and incurable vision loss.

Strabismus: A condition in which the eyes are not properly aligned with each other. When looking at an object, the images do not fall on corresponding retinal locations.

Visual Acuity: Refers to a measure of the spatial resolving capacity of the visual system.

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Review Article

Age-Related Neurodegeneration and Memory Loss in Down Syndrome

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Down syndrome (DS) is a condition where a complete or segmental chromosome 21 trisomy causes variable intellectual disability, and progressive memory loss and neurodegeneration with age. Many research groups have examined development of the brain in DS individuals, but studies on age-related changes should also be considered, with the increased lifespan observed in DS. DS leads to pathological hallmarks of Alzheimer's disease (AD) by 40 or 50 years of age. Progressive age-related memory deficits occurring in both AD and in DS have been connected to degeneration of several neuronal populations, but mechanisms are not fully elucidated. Inflammation and oxidative stress are early events in DS pathology, and focusing on these pathways may lead to development of successful intervention strategies for AD associated with DS. Here we discuss recent findings and potential treatment avenues regarding development of AD neuropathology and memory loss in DS.

1. Introduction

The most common cause of dementia is Alzheimer's disease (AD), with rates of prevalence increasing steadily from 60 years of age to reach almost 40% by the age of 85 [1]. AD is defined as the presence of neuritic plaques, which are composed of extracellular deposits of amyloid beta, and neurofibrillary tangles [2]. Neurodegeneration in the later stages of AD is widespread, with massive synapse loss and an overall decline in grey matter resulting from neuronal loss in cortical and hippocampal regions. Cortical neuronal loss is preceded by degeneration of certain subcortical neuronal populations, including basal forebrain cholinergic neurons (BFCNs) [3] and noradrenergic neurons of the locus coeruleus (LC-NE) [4, 5].

While the majority of AD cases are considered sporadic, mutations in amyloid precursor protein (APP) and presenilins 1 and 2 (PS-1 and PS-2) genes are responsible for most of the cases of AD considered "familial" [6]. These mutations lead to alterations in APP metabolism that result in an overabundance of amyloid plaques. Similarly, APP processing is also affected in Down syndrome (DS), a population who exhibit histopathology consistent with AD by the 4th and 5th decades of life with near uniformity, as well as increased risk for dementia [7, 8]. Located on chromosome 21, APP is triplicated in DS, and amyloid-beta deposition is frequently profound in these individuals [9–11]. Recently, cases of familial AD resulting from duplication of only the *APP* locus have been discovered [12], further defining a role for APP in AD dementia. However, few studies have been able to correlate plaque load with dementia severity. Rather, cognitive function correlates most strongly with the degeneration of cholinergic neurons in the basal forebrain. Reversal of cholinergic hypofunction in AD with choline acetyl transferase inhibitors has been shown to facilitate memory function, albeit to a moderate degree [13]. However, it is still not known what causes the cholinergic degeneration, or if other parallel factors also contribute to the disease. Some potential mechanisms include neuroinflammation, oxidative stress, amyloid toxicity, and abnormal phosphorylation of proteins including the microfilament-associated protein tau; etiological causes include genetic mutations, diet, sedentary

lifestyle, and environmental toxins [14]. While familial causes of AD are rare and idiopathic AD is difficult to model, DS presents a large and relatively homogenous population with relevant animal models that can serve to illuminate possible etiologies or treatment paradigms in AD.

In the current paper, we will discuss current theories regarding biological mechanisms and potential treatment paradigms for DS individuals with AD-like dementia (DSD). We include data from animal models, as well as from humans with DSD, and propose potential early prevention models for this difficult and progressive condition.

2. Down Syndrome: A Genetic Insight into AD

The uniformity with which individuals with DS acquire AD neuropathology makes this population important to study, not only to gain a better understanding of AD, but also because there are currently no effective treatment paradigms for DSD [8, 15]. Because they have physiological alterations in cardiac and metabolic systems, cholinesterase inhibitors may be contraindicated in some DSD patients [15, 16]. DS is the most common aneuploidy, occurring as frequently as approximately 1 in every 700 live births in the US [17]. DS results in variable levels of intellectual disability, along with congenital defects, and increased risk of certain cancers, such as leukemias [18]. As maternal age continues to increase and medical interventions have increased the lifespan of DS individuals, the prevalence of DSD continues to grow. The diverse and heterogeneous neurodegeneration in AD and in normal aging are accelerated in DS, and lessons learned from DSD patients may uncover therapeutic targets with widespread implications. In fact, DS can be considered a form of segmental progeroid syndrome, or accelerated aging [19, 20].

Studies assessing the effects of age on cognition in DS demonstrate a greater incidence of short-term memory impairment in DS individuals over 35 years of age, as well as increasing rates of dementia, aphasia, and agnosia [23] while detriments in executive function are evident already in adolescence [24]. As in idiopathic AD, DSD patients display dysfunction of language and motor skills, seizure onset, and behavioral abnormalities [25], in addition to AD-like pathology, including amyloid-beta deposits, neurofibrillary tangles, loss of BFCNs, and pathological alterations in mitochondria and endosomes [26–29]. While trisomy 21 constitutes the triplication of over 300 genes [30, 31], recent animal studies have sought to elucidate which genes may contribute to the observed neurodegenerative pathology. Based on genetic studies in mouse models of DS, several specific genes contained within the triplicated region of murine chromosome 16 (which corresponds to an equivalent section on human Chr. 21; see Figure 1) have been implicated in the DSD neuropathology. One of the most important genes associated with DS is the *amyloid precursor protein (APP)* gene—increased APP production may partially contribute to DSD-related oxidative stress as well as inflammation. Accumulation of amyloid-beta monomers can directly impair mitochondrial function resulting in energy depletion [32], and it is also well known that accumulation of amyloid—either in tissue culture or *in vivo*—leads to activation of inflammatory cascades [33, 34], most likely via both microvascular dysfunction and activation of resident glial cells in brain parenchyma. Furthermore, cortical DS neurons exhibit impaired mitochondrial function that results in reduced energy production and elevations in reactive oxygen species (ROS) [35]. Studies using the Ts1Cje mouse model for DS, which does not include triplication of the SOD or APP genes [36], suggest that other triplicated genes may be involved in mitochondrial abnormalities observed in DS. In addition, while *APP* and *SOD-1* each may contribute to the disease, neither gene is solely responsible for the degenerative changes that occur in DS [37]. Other genes located on the critical region include Ets-2 and DSCR1 (Figure 1), which have both been linked to neurodegeneration [35, 38]. In this paper, we will provide evidence, from our recent work and others, suggesting that inflammation and oxidative stress are early dysregulations which may be responsible for age-related dementia and associated pathology in DSD.

3. Modeling DS Pathology: The Ts65Dn Mouse

As discussed elsewhere in this issue, a spontaneous translocation of a portion of murine chromosome 16 onto chromosome 17 led to the formation of a DS model, the Ts65Dn mouse [39]. The translocated segment of chromosome 16, syntenic to a significant portion of human chromosome 21 (Figure 1), thus provided a genetic triplication which can be passed on to offspring [39]. Nearly 140 known genes are triplicated in Ts65Dn mice, of which 60% are also located on human chromosome 21 [40]. More importantly, these mice exhibit normal lifespans, allowing for the analysis of progressive neurodegenerative alterations. While Ts65Dn mice fail to develop amyloid plaques, they do exhibit elevated levels of APP and associated peptides in the hippocampus [41–43] and increased phosphorylation of tau protein [44, 45]. Ts65Dn mice also show increased inflammatory morphology with aging [22, 46] (see also Figure 2) synaptic dysfunction [47, 48], and a failure of neurotrophic signaling, particularly involving the retrograde transport of nerve growth factor (NGF) to the basal forebrain [42, 46, 49, 50], and downregulation of brain-derived neurotrophic growth factor (BDNF) levels [51, 52]. In addition, they exhibit age-related degeneration of LC-NE and BFCN neurons [22, 53–55]. Memory deficits are progressive in these mice and onset coincides with BFCN atrophy [43, 46, 56]. Interestingly, a study by Belichenko et al. [57] suggested that 33 genes, included in the so-called "DS critical region" (DSCR) of genes in humans, and triplicated in a novel mouse model (Ts1Rhr), might be responsible for many of the physiological and behavioral detriments observed in the Ts65Dn mice, narrowing the search for the set of genes involved in DSD neuropathology [57]. However, other studies have shown that although this "critical region" is necessary for cognitive impairment and pathology to develop [58], overexpression of these particular genes is not sufficient to generate DSD, at least not in mouse models, demonstrating the complex

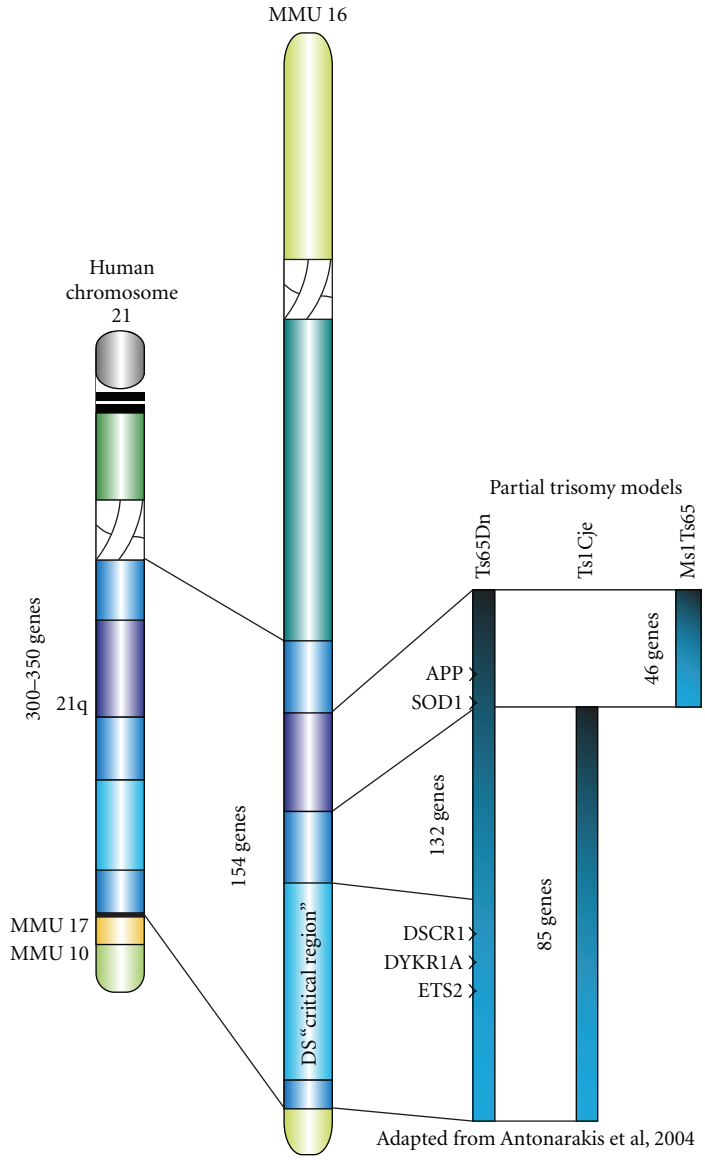


Figure 1: Mouse models for DS. Schematic of the gene segments involved in the so-called "Down syndrome critical region" (DSCR) in human chromosome 21, as well as in different mouse models of the condition. Note that the Ts65Dn mouse contains all genes included in the DSCR, as well as a set of 132 other genes including *SOD* and *APP*. Modified from Antonarakis et al. 2004 [21].

nature of DS-related dementia and neuropathology with aging.

While degeneration of basal forebrain cholinergic neurons (BFCNs) occurs during normal aging, DSD and AD are defined by rapidly accelerated loss of these projection neurons, and cholinergic dysfunction correlates strongly with the progression of cognitive decline in both diseases [59, 60]. Ts65Dn mice show consistent learning and memory deficits on spatial reference and working memory tasks [56, 61–67]. Most of these deficits become apparent between 4 and 12 months of age [56], suggesting, indeed, that the behavioral dysfunction developing in the Ts65Dn mouse mimics the segmental progeria syndrome observed in terms of brain function in humans with DS. Ts65Dn mice exhibit deficits in novel object tasks, which are reversed by the partial *N*-Methyl-D-aspartic acid (NMDA) glutamate receptor blocker Memantine (Namenda) [68–70]. These findings suggest that glutamate and GABA transmitter systems are affected by the genetic alterations in Ts65Dn, directly or indirectly, in Ts65Dn mice, something that has been suggested by work from other research groups as well [71, 72]. In a manuscript by Rueda et al. [71], they found that treatment with memantine in aged Ts65Dn mice improved spatial learning but did not affect the number of dentate granule cells, suggesting that the effects of memantine may be pharmacological, rather than neuroprotective. These data were further supported by our findings, that memantine increased working memory performance, particularly in

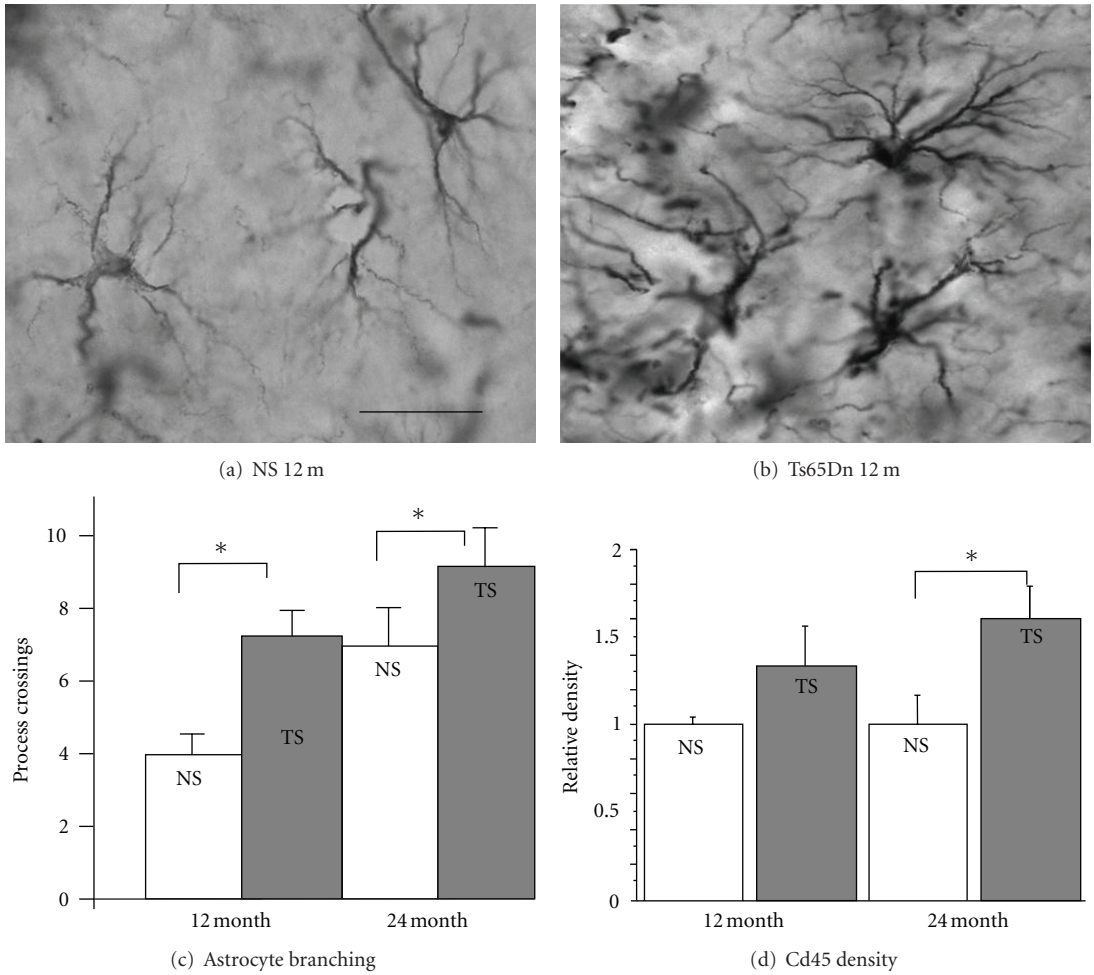


Figure 2: Gliosis in hippocampus of Ts65Dn mice. Brain tissue sections from Ts65Dn mice and age-matched normosomic littermates, showing typical hippocampal morphology of astrocytes, using the glial marker GFAP (a) and (b). The sections were from 12-month-old normosomic (NS, a) or Ts65Dn (TS, b) mice. Note increased number of astrocytes in TS mice, as well as elevated expression of GFAP and an activated morphology, with more branching and thicker branches in the TS compared to NS mouse. (c) Astrocyte branching measurements (GFAP labeling) in the hippocampus reveal increased branching in TS mice compared to NS age-matched controls, a sign of activation following inflammatory or other pathological processes. Astrocytosis is increased with aging in the TS mice to a greater extent than in NS mice. (d) Density of a marker for microglial cells, Cd45, is also increased with age in Ts65Dn (TS) but not in age-matched normosomic (NS) mice, indicating ongoing microglial activation in this brain region. Inset in (b) represent 100 microns. *Data were not published previously*.

a novel object task, but did not rescue hippocampal, cholinergic, or locus coeruleus neurons from progressive neurodegeneration [70]. The cognitive impairment observed over time in Ts65Dn mice parallels cognitive impairment in adult DS individuals with early or moderate AD, tested on the WISC-R behavioral battery, showing progressive deterioration in executive function, comprehension, picture completion, vocabulary, and digit span [73]. The memory deficits indicate hippocampal and frontal cortex dysfunction and together with septohippocampal degeneration indicate that the Ts65Dn mouse is a unique model to understand the progression of neuropathology and memory loss in DSD.

4. Locus Coeruleus Degeneration in DSD

LC-NE degeneration, while less studied than BFCN loss, is another hallmark of AD [74]. NE neurotransmission exerts effects on neurons, glia, and blood vessels throughout the neuraxis. LC-NE lesions, using the selective NE neurotoxin DSP-4, give rise to aggravated amyloid accumulation, oxidative stress, and memory loss in transgenic AD models [75–77]. Findings suggest that LC-NE effects are mediated both directly, via neurotransmission changes in the limbic system, and indirectly, via aggravation of amyloid accumulation, inflammation, and oxidative stress pathways. NE-mediated neuroprotection of oxidative stress on BCFNs *in vitro* is independent of adrenergic receptor activation or intracellular accumulation, [78] suggesting a role for NE in the neutralization of hydroxyl radicals. The antioxidant activity of NE provides a pharmacological link between LC-NE and cholinergic survival. NE circuitry also exhibits a direct influence on memory formation. BFCNs activity is modulated by NE via adrenergic receptor activity [79],

and pharmacological stimulation of NE receptors leads to improved cognitive performance both in rodent models and in humans [80]. While NE is an essential modulator of memory through its ability to regulate synaptic mechanisms, NE depletion is not sufficient to significantly alter memory function in intact animals [22]. Yet, NE depletion in the presence of cholinergic dysfunction exacerbates memory impairments [22] and may therefore aggravate deficits in memory systems dependent on the basal forebrain cholinergic neurons. In a recent study, Ts65Dn and NS mice were lesioned using the NE neurotoxin DSP-4 at 4 months of age and were then studied at 8–10 months of age in terms of behavior and neurochemistry. As can be seen in Figure 3 and in [22], the NE lesion gave rise to a significant aggravation of both memory loss and neuropathology in Ts65Dn but not in NS mice, including degeneration of hippocampal and BFCNs as well as increased inflammatory markers. These findings suggest that NE neurotransmission, albeit important for normal function of the brain, plays a particularly important role for curbing age-related pathology in the form of inflammation and neuronal loss. This notion has been supported by other investigators, showing enhanced effects of DSP-4 lesions in APP transgenic mice [76, 81, 82]. These investigators also found that administration of the NE precursor L-threo-DOPS restored microglial functions in NE-depleted mice [76], suggesting a reciprocal system where the amyloid cascade, inflammatory markers, and NE innervation systems affect each other. Interestingly, others have also shown that LC neurons spontaneously degenerate in AD mouse models [83], again suggesting a specific link between accelerated amyloid accumulation and degeneration of LC neurons.

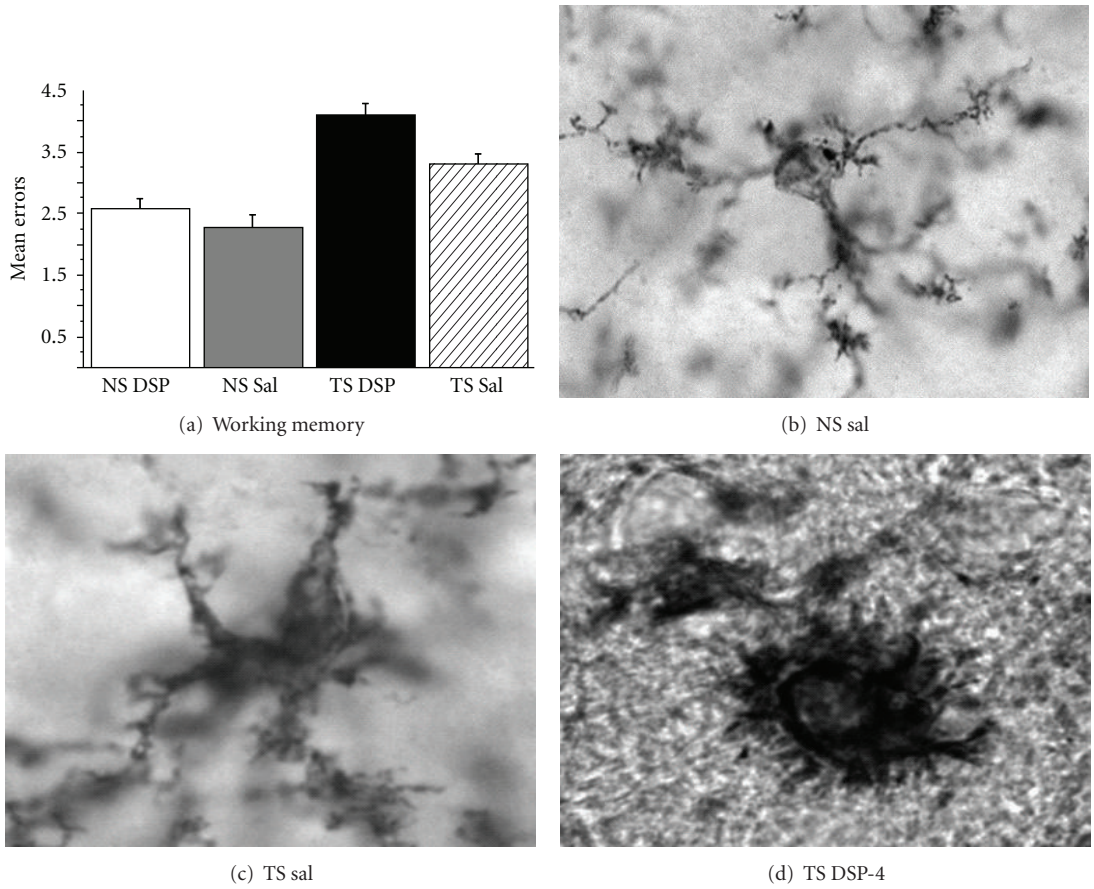
Importantly, individuals with DSD exhibit early and progressive degeneration of LC-NE neurons [84]. Recently, a study by Salehi et al. [55] demonstrated successful recovery from memory loss in Ts65Dn mice using the NE precursor Droxidopa (L-threo-dihydroxyphenylserine). These results are promising and should be considered in future clinical treatment paradigms for DSD patients. Since LC-NE degeneration is common to both Parkinson's disease (PD) and AD patients [85–87], future pharmaceutical interventions for dementia may include enhancement of NE neurotransmission also for these neurological conditions. Promising clinical pilot studies have already been initiated in terms of the NE reuptake inhibitor Atomoxetine and memory loss in PD [88] and in Alzheimer's disease [89, 90] even though much remains to be done in terms of incorporating NE enhancement treatment for dementia. LC-NE neurons partake in the regulation of blood vessels, microglial cells, as well as neurons, and degeneration of this monoaminergic cell group can be an active player in neuropathological processes in age-related dementia of different etiology.

5. Inflammatory Pathology in AD and DSD

As in AD, individuals with DSD consistently exhibit chronic inflammation in limbic system areas of the brain, with increases in microglial and astrocytic activation coupled with IL-1β and TNF-α cytokine release [91–93]. Microglial activation typically arises in the entorhinal cortex before developing in the hippocampus and surrounding cortex as well as the basal forebrain [26, 27]. BFCNs are highly sensitive to inflammation and oxidative stress [94], but specific biological mechanisms for their selective loss in AD and in DSD have not been revealed. There is also evidence that TNF-α-induced cortical inflammation at cholinergic terminals leads to retrograde degeneration of BFCNs [95]. Recent work suggests that inflammation due to loss of noradrenergic innervation from the LC-NE innervation of BFCNs is a plausible explanation for the selective vulnerability of these neurons in DSD and AD [22]. β-adrenergic receptors are expressed in astrocytes and microglia and modulate the cytokine release [96]. The reduction of noradrenergic neurons in the LC correlates with amyloid plaques and dementia severity in AD [97, 98]. NE treatment of cholinergic cells *in vitro* reduces expression of IL-1β and TNF-α, as well as proinflammatory proteins such as iNOS [96]. Since Ts65Dn mice exhibit significant degeneration of both BFCNs and LC-NE neurons, it is not surprising that we found accelerated and age-related astrocytosis and microgliosis in the hippocampus of this mouse model of DS (Figures 2 and 3). As mentioned above, depletion of noradrenergic terminals in murine models of AD results in increased inflammatory cytokine production, activated microglial morphology, and amyloid deposition [76, 82, 99]. NE terminal destruction also impeded cholinergic neurotransmission in AD models which otherwise show no cholinergic deficits [81]. Thus, while inflammation may affect many of these neurodegenerative processes, it also can increase in response to early abnormalities in ACh and NE signaling, since there is a reciprocal relationship between neuronal and glial modulation of inflammatory processes, especially during neurodegenerative disease [96]. Based on these studies, it is difficult to determine whether BFCN and LC-NE degeneration activates the inflammatory pathways, or if the cytokine production by astrocytes and microglia, in turn, causes the neuronal degeneration in DSD and AD. Most likely, all of these processes have interactive and escalating effects on each other, leading, in the end, to memory loss and AD pathology.

6. Neurotrophic Factors and DS

The survival and maintenance of BFCNs depend on neurotrophic support from NGF and BDNF [100]. NGF mRNA is expressed at high levels in regions innervated by cholinergic terminals, such as the neocortex, dentate gyrus, and the hippocampal pyramidal layer [3]. Upon release from postsynaptic neurons, NGF binds to its high-affinity receptor, TrkA, on BFCN nerve terminals, initiating receptor oligomerization which leads to signaling cascades through PI3K and ERK activation and endocytosis of the ligand-receptor complex [101]. This complex is retrogradely transported to the soma where it facilitates signal transduction of phenotypic markers such as choline acetyltransferase [101, 102]. Exogenous administration of NGF rescues BFCNs from age- or toxin-related degeneration and reverses cognitive dysfunction in animal models of AD or normal aging [103].



(a) Working memory

(b) NS sal

(c) TS sal

(d) TS DSP-4

Figure 3: Effects of the NE neurotoxin DSP-4 on Ts65Dn and normosomic mice. Note significant aggravation of performance in a memory task (a) coupled with aggravated activation of microglial cells (b–d) in the hippocampal formation, as evidenced by Cd45 immunohistochemistry. (a) Average number of errors in a water radial arm maze. The NE lesion exhibited more pronounced effects on errors in the maze in TS than in NS mice, and TS mice performed more errors than NS mice, regardless of NE lesions (DSP) or not (Sal). (b–d) Cd45 staining of microglial cells in the hippocampus in a normosomic mouse (NS) treated with saline (b), a Ts65Dn mice on saline (c), and a Ts65Dn mouse that received DSP-4 lesions of the LC-NE neurons (d). Note significant activation of individual microglial cells as a result of the NE lesion in TS mice compared to controls. Quantitation of inflammatory processes is available in Lockrow et al., 2011 [22].

While the production of NGF in the hippocampus and cerebral cortex has been shown to be unaltered or even increased in AD [103], NGF levels in the basal forebrain exhibit significant decline [104]. A compensatory increase in NGF expression in target regions may be due in part to loss of TrkA receptor expression in BFCN neurons, which occurs early in AD and is recapitulated in aged rodents [3, 105]. Murine models for DS show reductions in retrograde NGF trafficking which occurs in part due to enlarged, dysfunctional endosomes [42, 49, 101]. Recent studies have shown that these endosomal changes can be caused by overexpression of APP [42, 106]. Abnormal endosomes are present in both AD and DSD brains [29] and localize to the vulnerable regions such as the basal forebrain and the hippocampus [107] suggesting that endosomal trafficking of NGF linked to TrkA may be a pathological pathway to explore further in DSD brains.

BDNF also promotes BFCN survival and cholinergic signaling [108–110]. BDNF expression is reduced in AD [109], and BDNF levels are reduced in serum from DS individuals [111], and in brain tissue from the Ts65Dn mouse model for DS [52], and has been shown to be linked to memory function, as well as synaptic plasticity and neurogenesis [112]. BDNF expression is increased following exercise and may therefore contribute to the beneficial effects of voluntary exercise observed in AD as well as in normal aging in humans and animal models [113–117]. Interestingly, several studies have shown that LC-NE innervation into cortical regions regulates the expression of BDNF, suggesting a close link between loss of BDNF expression and LC-NE degeneration in DS [118]. In a recent manuscript by Counts and Mufson [119], the authors demonstrated that administration of NE protected cultured neurons from amyloid-beta-mediated toxicity by upregulating both NGF and BDNF expression. Further, the authors found that NE inhibited increased reactive oxygen species (ROS) and caspase activation caused by the neurotoxin, suggesting also a direct link between the neurotrophic factors, NE innervation, and oxidative stress.

Treatment with functional blocking agents for NGF and BDNF removed the beneficial effects, indeed suggesting that NE effects were mediated by the trophic factors. This paper therefore linked several pathological processes in DSD and AD, providing direction for future research and treatment options. Our recent study using Ts65Dn mice extended these findings *in vivo*, by showing that an LC-NE lesion, using the neurotoxin DSP-4, decreased BDNF expression in frontal cortex, a region associated with working memory loss in the Ts65Dn mouse model [22]. We also found a significant correlation between BDNF expression and NE levels, as well as between BDNF expression and working memory errors, suggesting a clear link between BDNF expression and memory function dependent on this region. BDNF and NGF have been associated with neuroprotection against oxidative stress in neurons [119, 120], suggesting that DSD patients may exhibit increased sensitivity to oxidative stress because of reduced expression of these neurotrophic factors.

7. Oxidative Stress and DSD Pathology

Individuals with DS exhibit elevated oxidative stress early in life [121]. Oxidizing free radicals, also known as ROS, are cytotoxic byproducts of normal mitochondrial metabolism and are normally processed by endogenous antioxidants. But when levels of mitochondrial ROS production exceed the intracellular antioxidant defenses, oxidative molecules can disrupt cellular functions, negatively affecting synaptic plasticity and eventually leading to neuronal injury and apoptosis [122]. The hippocampal formation exhibits a high vulnerability to both ischemic and neurotoxic injury associated with oxidative stress [123]. A marker of RNA oxidative damage, 8-hydroxyguanosine (8-OHG), is elevated in neurons of the hippocampus and cortex early in the progression of AD and precedes much of the pathology in these regions, suggesting that oxidative stress may be the earliest event in AD-related disease processes [124]. Postmortem analysis revealed that 8-OHG immunoreactivity increased significantly in cortical neurons of DS individuals in their teens and twenties, while amyloid-beta burden was increased only after 30 years of age [125], strongly suggesting that oxidative stress is an early event also in DS. The central question is why is oxidative stress so rampant in the brain of DS individuals?

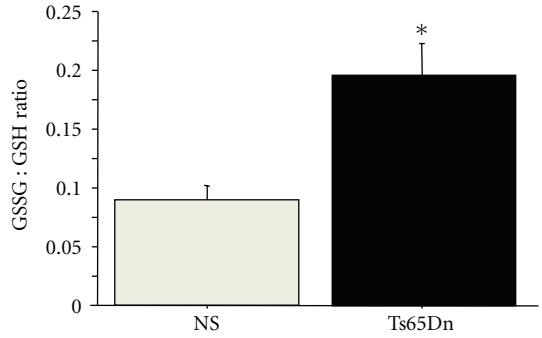
Part of the answer to that question may be the triplication of both *APP* and *SOD-1* genes in DS (Figure 1). The balance between ROS production and the scavenger enzyme pathways is tightly regulated in the cell during normal conditions. We propose that the increase in expression of SOD-1 in DS leads to a reduction in superoxide but an increase in the accumulation of hydrogen peroxide (H_2O_2) in tissues. This hypothesis is based on a superarray using pooled samples of tissue from the hippocampus of Ts65Dn mice revealing significant elevations in hippocampal SOD-1 expression with only a moderate increase in the other scavenger enzymes, including glutathione reductase and catalase (Figure 4). Elevated rates of conversion from superoxide to H_2O_2 would lead to lipid peroxidation in neurons and glia, accumulating with time, and leading to the neuropathology observed in Ts65Dn mice with age, as well as in DS individuals. This hypothesis was recently validated by studies from Harris-Cerruti et al. [37], showing that a mouse model consisting of double SOD-1/APP overexpression leads to memory loss and neuropathology, as well as elevated ROS in the brain, while APP overexpression alone was less effective in generating neurodegeneration or ROS accumulation. When the investigators examined hippocampal slices for long-term potentiation (LTP), they found that LTP was impaired in both tg-SOD and tg-APP-SOD mice, but not in tg-APP mice, suggesting that the APP overexpression alone did not affect this cellular component of hippocampal plasticity. SOD-1 overexpression alone also gave rise to ROS accumulation, but not to the extent observed in APP/SOD-1 overexpression mice, suggesting a comodulation of oxidative stress pathways by the *APP* and *SOD-1* genetic overexpression [37].

There is a controversy in the literature regarding beneficial or damaging effects of SOD overexpression. While some investigators show that SOD-1 or SOD-2 overexpression rescues neuropathology in AD transgenic mouse models [126], others demonstrate aggravated pathology when overexpressing SOD-1 [126], suggesting that there is a complicated relationship between SOD-1 and SOD-2 function in the CNS. Gardner and colleagues [127] investigated this question using a minimal mathematical model. The authors concluded that the outcome depended on a balance between processes consuming superoxide without forming H_2O_2 and those consuming superoxide with high H_2O_2 yield [127]. Our investigations shed some light on this particular question for DS brains, since Ts65Dn mice exhibited elevated expression of both glutathione and catalase (Figure 4), presumably as a response to elevated H_2O_2 levels in the brain. However, since most investigators use indirect methods of measuring H_2O_2, such as measuring lipid peroxidation or associated markers, it has not been shown, at least not to our knowledge, whether neurons or glia from DSD patients or Ts65Dn mice exhibit elevated H_2O_2 levels, even though studies of postmortem brain tissue have shown that levels of peroxiredoxin, which is an enzyme involved in eliminating H_2O_2, are elevated in both DSD and AD [128]. The role of oxidative stress in development of pathology in DS individuals is further discussed in other sections of this issue.

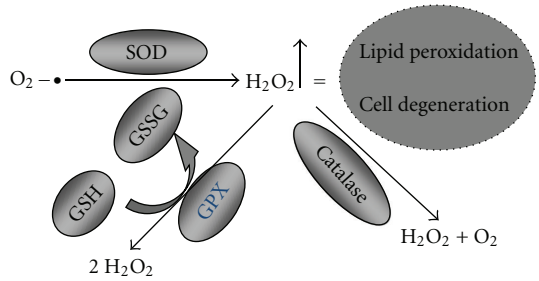
Early increases in ROS suggest that antioxidant therapy may benefit DS individuals with AD pathology. While clinical results for vitamin E treatment in AD patients have been mixed to this point [129], there have been minimal studies to determine whether antioxidants could be beneficial in DSD, despite a recent study of vitamin E administration during childhood in DS [130]. We recently reported beneficial effects of long-term vitamin E treatment in Ts65Dn mice [131] and suggest that this may be a viable future option for DSD. Ts65Dn mice were given vitamin E in their diet from 4–10 months of age, and cognitive performance was tested, followed by brain pathology. BFCN and hippocampal cell loss were reduced significantly, and neuroinflammation associated with microglial activation was also significantly reduced, suggesting a strong connection between inflammatory and oxidative stress pathways [131]. Oxidative stress measures correlated with improved cognitive

Age	4 mo	8 mo	4 mo	13 mo
Catalase	↑ 35%	↑ 21%	↑ 35%	↑ 24%
GPX 1	↑ 17%	↑ 7%	↑ 44%	↑ 21%
GPX 3	↓ 9%	↑ 40%	↑ 29%	↑ 64%
iNOS	↑ 22%	↑ 101%	↑ 38%	↑ 162%
COX-1	↑ 10%	↑ 5%	↑ 50%	↑ 17%
COX-2	↑ 10%	↑ 8%	↑ 51%	↑ 6%
SOD-1	↑ 76%	↑ 70%	↑ 54%	↑ 73%
SOD-2	↓ 8%	↓ 29%	↑ 20%	↑ 1%
Vimentin	↑ 23%	↑ 5%	↑ 20%	↑ 18%
AChE		↑ 8%	↓ 15%	
APP		↑ 52%	↑ 28%	
ChAT		↓ 32%	↓ 47%	

(a) Hippocampal gene expression



(b) Oxidized glutathione in cortex at 4 mo



Key: superoxide dismutase (SOD), reduced glutathione (GSH), oxidized glutathione (GSSG), glutathione peroxidase (GPX)

(c) Scavenger pathway

Figure 4: (a) Superarray (SABiosciences, Frederick, MD) against oxidative stress and inflammatory markers was used on hippocampal tissue from pooled samples (3 per group) of Ts65Dn and Normosomic mice at 4, 8, 10, and 13 months of age. Note the increased expression in *APP* and *SOD-1* due to increased gene dosage of these genes. However, glutathione peroxidase 1 and 3 (GPX 1 and 3), as well as catalase levels, were not increased to the same extent. Further investigation of the glutathione enzymatic pathway revealed increased GSSG:GSH ratio in Ts65Dn compared to normosomic brain (b), suggesting, a compensatory processing of free radicals, but not sufficient to eliminate peroxidation in neurons. Glutathione exists in two forms: GSH (reduced form) and GSSG (oxidized form). Normally the relationship between these two forms is 1 : 10 in healthy cells. (c) Schematic representation of the ROS scavengers, demonstrating that elevated SOD levels may lead to increased H_2O_2 levels, leading to enhanced stress on the glutathione and catalase pathways. *Data were not published previously.*

performance, supporting the hypothesis that oxidative stress plays an important role for memory loss associated with DSD. Based on these encouraging findings, and the relatively minor risks associated with vitamin E treatment, we would suggest future development of this treatment paradigm for individuals with DS as a prevention strategy.

8. Overexpression of APP: Disease Modifier

An involvement of the amyloid cascade in the progressive memory loss and neuropathology in DS cannot be denied. It is likely that the overproduction of APP in DS individuals (Figure 1) converges upon both oxidative stress and inflammation pathways in the brain, to cause added harm to the DSD patient with time. Amyloid-beta-induced oxidative stress appears to be mediated through an NMDA receptor-mediated increase in Ca^{2+} influx [132]. Elevated intracellular Ca^{2+} disrupts mitochondrial function [133] and may explain the reduced mitochondrial efficiency seen in AD. As previously shown by our laboratory, Ts65Dn mice have deficits in expression of calbindin, a neuronal calcium-binding protein, in the hippocampus [46], suggesting further dysregulation of intracellular Ca^{2+} pathways. It is also possible that other genetic components of the triplicated gene segment aggravate DS-related AD pathology. The regulator of calcineurin 1 (RCAN1 or DSCR1) is also over-expressed in DS and in Ts65Dn mice (Figure 1). A recent manuscript by Porta et al. [134] demonstrated that RCAN1 knockout neurons ($RCAN1^{-/-}$) exhibited a reduced response to oxidative stress, and the investigators therefore suggested vulnerability to oxidative stress downstream from the SOD-1-mediated accumulation of H_2O_2 in DS and in AD. These findings are important for continued efforts in

determining the role of different genes in DS to provide additional substrates for neuroprotection strategies.

9. Outstanding Questions

Outstanding questions in this field should focus on prevention and/or treatment options for DSD. As individuals with DS live longer and medical interventions have been able to modify cardiovascular problems or other health issues, the incidence of DSD will go up dramatically in the next couple of decades. Based on recent findings related to vitamin E and antioxidant capacity, we feel that it is important to assess prevention in DS individuals at an early stage using vitamin E and/or other antioxidants. Further, treatment with NE enhancing drugs, such as Atomoxetine (Strattera) [88, 135], has shown promising results in children with ADHD and in PD; it is possible that these pharmaceutical interventions may be beneficial for working memory deficits and early onset problems with executive function in persons with DSD as well. It is important to note that several disease processes, related to inflammation, oxidative stress, cholinergic cell loss, calcium homeostasis, amyloid accumulation, and locus coeruleus degeneration, all converge on the progressive deficits observed in the limbic system of individuals with DS with age. Combination therapy targeting several aspects, or working upstream from the observed pathology, should therefore be developed. Finally, a national registry for DSD and age-matched control brain tissue and associated tissues is long overdue. The development of such a repository will allow centralized and streamlined studies into etiology but also possible treatment paradigms for DSD and finally render this field well-deserved attention, using a nation-wide collaboration for DSD-related studies.

Glossary

(i) Alzheimer's disease (AD): the most common form of dementia.

(ii) Down syndrome (DS): whole or segmental triplication of chromosome 21 in humans.

(iii) Basal Forebrain cholinergic neurons: small group of neurons in basal forebrain carrying acetylcholine as their transmitter, and innervating large portions of the CNS.

(iv) Locus coeruleus noradrenergic neurons (LC-NE): small population consisting of a few thousand neurons in humans, located in the brainstem and innervating most portions of the brain and spinal cord.

(v) Amyloid beta: cleavage form of amyloid precursor protein that accumulates in the brain of people with AD and DSD and has both inflammatory and oxidative stress effects on neurons.

(vi) Proinflammatory cytokines: small molecules that are released either in the blood or directly in the brain by inflammatory cells and contribute to inflammatory damage in the brain.

(vii) Oxidative stress scavengers: a set of enzymes, including superoxide dismutates, catalase, and glutathione, that reduce free radicals to water via a set of enzymatic reactions.

(viii) Long-term potentiation (LTP): a form of cellular potentiation of specific processes often used for studies of cellular learning and memory mechanisms.

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Research Article

Polymorphisms in *HSD17B1*: Early Onset and Increased Risk of Alzheimer's Disease in Women with Down Syndrome

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Background/Aims. Genetic variants that affect estrogen activity may influence the risk of Alzheimer's disease (AD). In women with Down syndrome, we examined the relation of polymorphisms in hydroxysteroid-17beta-dehydrogenase (*HSD17B1*) to age at onset and risk of AD. *HSD17B1* encodes the enzyme 17β-hydroxysteroid dehydrogenase (HSD1), which catalyzes the conversion of estrone to estradiol. *Methods*. Two hundred and thirty-eight women with DS, nondemented at baseline, 31–78 years of age, were followed at 14–18-month intervals for 4.5 years. Women were genotyped for 5 haplotype-tagging single-nucleotide polymorphisms (SNPs) in the *HSD17B1* gene region, and their association with incident AD was examined. *Results*. Age at onset was earlier, and risk of AD was elevated from two- to threefold among women homozygous for the minor allele at 3 SNPs in intron 4 (rs676387), exon 6 (rs605059), and exon 4 in *COASY* (rs598126). Carriers of the haplotype TCC, based on the risk alleles for these three SNPs, had an almost twofold increased risk of developing AD (hazard ratio = 1.8, 95% CI, 1.1–3.1). *Conclusion*. These findings support experimental and clinical studies of the neuroprotective role of estrogen.

1. Introduction

The neurotrophic and neuroprotective mechanisms of estrogen have beneficial effects on brain function that include increases in cholinergic activity [1–5], antioxidant activity [6, 7], and protection against the neurotoxic effects of beta amyloid [8–11]. Thus, the dramatic declines in estrogen following menopause may contribute to higher risk of AD in women [12].

Allelic variation in genes within the estrogen biosynthesis and estrogen receptor pathways may modify cerebral estrogen activity and influence risk of AD. The hydroxysteroid-17beta-dehydrogenase (*HSD17B1*) gene, located on chromosome 17q11-q21, encodes the enzyme 17β-hydroxysteroid dehydrogenase (HSD1), which catalyzes the conversion of estrone to estradiol. Variants in *HSD17B1* have been examined for their relation to hormone levels, [13, 14] breast cancer [13, 15–24], endometriosis and endometrial cancer [13, 25–29], colorectal cancer [30, 31], and prostate cancer [32], with inconsistent results. Studies of polymorphisms in *HSD17B1* have focused on rs605059, a nonsynonymous single-nucleotide polymorphism in exon 6. The T/C

polymorphism in rs605059, the change in bases at codon 313 in exon 6, is expressed as the change in amino acids from serine to glycine. The rs605059 SER313GLY variant has been associated with a modestly increased risk of endometriosis, estrogen receptor-negative tumors in breast cancer patients, and colorectal cancer in women, conditions known to be associated with estrogen regulation [16, 28, 29, 31], but not all studies have found positive associations. Along with two other haplotype-tagged SNPs, (rs676387 and rs598126), these common variants represent over 80% of the variation at this locus. [13]. Expression of *HSD17B1* was found to be increased in prefrontal cortex in late-stage AD [33], but variants in *HSD17B1* have not been examined for their association with age at onset or risk of AD.

Women with Down syndrome (DS) are at high risk for AD, with the onset of dementia 10–20 years earlier than women in the general population [34–36]. Early age at menopause and low levels of bioavailable estradiol in postmenopausal women with DS are both associated with earlier onset and increased cumulative incidence of AD [37, 38], suggesting that the decline in estrogen contributes to pathological processes leading to AD in this high-risk population. In this study, we examined the relationships between single-nucleotide polymorphisms in *HSD17B1*, age at onset, and cumulative incidence of AD in women with DS to determine if genotype was related to risk.

2. Materials and Methods

2.1. Subjects. The initial cohort included a community-based sample of 279 women with DS. Of these 279 women, 252 (90.3%) agreed to provide a blood sample, and 244 (96.8%) were genotyped for *HSD17B1*. All individuals were 30 years of age or older at study onset and resided in New York, New Jersey, Pennsylvania, or Connecticut. In all cases, a family member or correspondent provided informed consent, including blood sampling and genotyping, with participants providing assent. The distribution of level of intellectual disability and residential placement did not differ between participants and those who did not participate. Recruitment, informed consent, and study procedures were approved by the Institutional Review Boards of Columbia University Medical Center and the New York State Institute for Basic Research in Developmental Disabilities.

2.2. Clinical Assessment. Assessments were repeated at 14–18-month intervals over five cycles of data collection and included evaluations of cognition and functional abilities, behavioral/psychiatric conditions, and health status. Cognitive function was evaluated with a test battery designed for use with individuals with DS varying widely in their levels of intellectual functioning, as described previously [39]. Structured interviews were conducted with caregivers to collect information on changes in cognition, function, and adaptive behavior. Past and current medical records were reviewed for all participants using a standardized protocol.

2.3. Classification of Dementia. This is a longitudinal cohort study of onset of AD in women with Down syndrome. The classification of dementia status, dementia subtype, and age at onset was determined during consensus case conferences where information from all available sources was reviewed. Classifications were made blind to *HSD17B1* genotype. We classified participants into two groups, following the recommendations of the AAMR-IASSID Working Group for the Establishment of Criteria for the Diagnosis of Dementia in Individuals with Developmental Disability [40]. Participants were classified as nondemented if they were without cognitive or functional decline, or if they showed some cognitive and/or functional decline that was not of significant magnitude to meet dementia criteria ($n = 164$). Participants were classified as demented if they showed substantial and consistent decline over the course of follow up for at least one-year duration and had no other medical or psychiatric conditions that might mimic dementia ($n = 80$). Age at meeting criteria for dementia was used to estimate age at the onset of dementia. Of the 80 participants with dementia, three had a history of stroke or TIA and were excluded from the analyses. Three additional participants were also excluded because their findings suggestive of dementia may have been caused by another non-AD medical or psychiatric condition, leaving 164 nondemented and 74 demented women in the analysis. Only women with probable or possible AD were included in the dementia group for analysis.

2.4. DNA Isolation and Genotyping. Genomic DNA was extracted from peripheral blood leukocytes using the FlexiGene DNA kit (Qiagen). Isolation of DNA and genotyping were performed blind to the dementia status of the participant. We analyzed 4 single-nucleotide polymorphisms (SNPs) in *HSD17B1* and one flanking SNP (rs598126) in CoA synthase (COASY), which is in high-linkage disequilibrium with rs605059. These included rs605059 (SER313GLY, C > T), which has been the SNP most consistently and strongly associated with estrogen-related disorders. Additional tagging SNPS were selected to provide coverage of the gene or to include SNPs which had also been associated with estrogen-related disorders in at least one study. These included rs2830 (T > C), rs2676530 (G > A), rs676387 (G > T), and rs598126 (C > T). Table 2 provides the locations and allele frequencies of these SNPs. SNPs were genotyped using TaqMan PCR assays (Applied Biosystems) with PCR cycling conditions recommended by the manufacturer, and by Prevention Genetics using proprietary array tape technology. Accuracy of the genotyping (≥97%) was verified by including duplicate DNA samples by comparing the TaqMan and array tape data with results of restriction digestion polymorphisms (RFLPs) for several of the SNPs, and by testing for Hardy-Weinberg equilibrium. Not all genotypes were available for all women at all SNPs, so the numbers examined vary slightly by SNP.

2.5. Apolipoprotein E Genotypes. *APOE* genotyping was carried out by PCR/RFLP analysis using *Hha*I (*Cfo*I) digestion of an *APOE* genomic PCR product spanning the polymorphic (cys/arg) sites at codons 112 and 158, followed by acrylamide gel electrophoresis to document the restriction fragment

sizes [41]. Participants were classified according to the presence or absence of at least one *APOE* ε4 allele.

2.6. Potential Confounders. Potential confounders included level of intellectual disability, body mass index (BMI), ethnicity, and the presence of an *APOE* ε4 allele. Level of intellectual disability was classified as mild to moderate (IQ from 35 to 70) or severe to profound (IQ < 34), based on IQ scores obtained before the onset of AD. BMI was calculated as weight in kilograms divided by the squared height in square meters (kg/m^2) and was measured at each evaluation. The baseline measure of BMI was used in the analysis and was included as a continuous variable. Ethnicity was categorized as white or nonwhite.

2.7. Statistical Analysis. Prior to association analysis, we tested all SNPs for Hardy-Weinberg Equilibrium using the HAPLOVIEW program [42], and all were found to be in Hardy-Weinberg equilibrium. SNPs were analyzed with a dominant model in which participants homozygous for the common allele were used as the reference group, with the exception of rs605059 and rs598126. We coded the C allele at rs605059 as the high-risk allele since previous work had shown that women carrying the C allele at rs605059 had lower levels of estradiol and a lower estradiol/estrone ratio than women carrying the TT genotype [13]. We coded the C allele as the high-risk allele in rs598126 since previous work had shown the TT genotype to be associated with increased risk of breast cancer [15]. To code the remaining genotypes, we used common alleles for *HSD17B1* SNPs for Hapmap whites at the NCBI SNP web site (http://www.ncbi.nlm.nih.gov/projects/SNP/). In preliminary analyses, the X^2 test (or the Fisher's exact test when any cell had <5 subjects) was employed to assess the association between AD and SNP genotypes as well as other possible risk factors for AD including ethnicity, level of intellectual disability, and the presence of an *APOE* ε4 allele. Analysis of variance (ANOVA) was used to examine BMI and age by AD status.

The analysis was structured as a longitudinal cohort study of the onset of AD. We used Cox proportional hazards modeling to assess the relationship between *HSD17B1* genotypes, age at onset, cumulative incidence, and the hazard ratio of AD, adjusting for ethnicity, BMI, level of intellectual disability and the presence of an *APOE* ε4 allele. The time to event variable was age at onset for participants who developed AD and age at last assessment for participants who remained nondemented throughout the follow-up period. Because a set of three contiguous SNPs that span ~10 kb-rs676387, rs605059, and rs598126 were significantly associated with AD, we performed a haplotype analysis to identify haplotype(s) that may harbor a susceptibility variant(s) as implemented in the PLINK program [43]. For nearly all individuals, we were able to identify the most likely haplotypes from the genotype data with a high degree of certainty (i.e., the posterior probability approaching 1.0 for 91% of the cohort with the rest exceeding probability >0.7). Subsequently, we used the estimated haplotype as a "super-locus" (analogous to a microsatellite marker) to perform Cox proportional hazards modeling. We restricted the analysis to individuals with a posterior probability of carrying the haplotype of 1.0.

Table 1: Demographic characteristics.

Characteristic	Nondemented	Alzheimer's disease
N	164	74
Age at baseline (M, SD)**	47.3 ± 6.9	54.2 ± 6.7
Level of intellectual disability (*n*, %)		
Mild/moderate	97 (59.1)	35 (47.3)
Severe/profound	67 (40.9)	39 (52.7)
Ethnicity (*n*, %)		
Non-hispanic white	142 (86.6)	68 (91.9)
Nonwhite	22 (13.4)	6 (8.1)
Body mass index (M, SD)**	29.9 ± 6.7	28.0 ± 6.0
Apolipoprotein E ε4 allele (*n*, %)	34 (21.0)	20 (27.0)

** $P < 0.05$.

3. Results

3.1. Demographic Characteristics. The mean age of participants at baseline was 49.4 years (range 31.5 to 78.1), and 88 percent of the cohort were white. The mean length of follow-up was 4.5 (SD ± 2.4) years. Table 1 presents the demographic characteristics of the participants according to AD status. Participants who developed AD over the follow-up period were significantly older at baseline than nondemented participants (54.2 versus 47.3 years) and were more likely to have severe or profound level of intellectual function (52.7% versus 40.9%), but did not differ in the distribution of ethnicity or the frequency of the *APOE* ε4 allele. Women who developed AD had a significantly lower BMI at baseline than women who remained nondemented over the follow-up period. The mean age at onset of AD was 55.7 ± 6.4 years.

3.2. Analysis of SNPs in HSD17B1. Table 2 shows the locations and minor allele frequencies (MAFs) of *HSD17B1* SNPs for Hapmap whites at the NCBI SNP web site (http://www.ncbi.nlm.nih.gov/projects/SNP/) and for our cohort of women with DS. Allele frequencies were similar in women with DS to those observed in women without DS in the general population. Table 3 presents the distributions of *HSD17B1* genotypes and the association between *HSD17B1* SNPs and the hazard ratio of AD among women with Down syndrome, adjusted for age, ethnicity, level of intellectual disability, BMI, and the presence of an *APOE* ε4 allele. All of the 5 SNPs examined were in high-linkage disequilibrium (LD > 0.9, Figure 3). Three SNPs, rs676387, rs605059, and rs598126, showed significant associations with AD, the strongest being with rs605059. Women who carried one or two copies of the T allele at rs605059 were two to three times more likely to develop AD than women homozygous for the C allele (HR = 2.0, 95% CI, 0.98–4.2 for those with the CT genotype and HR = 3.0, 95% CI, 1.4–6.8 for those with the TT genotype) (Table 3) and had both earlier onset and higher

Table 2: *HSD17B1* SNP chromosomal location[a].

SNP	Chromosome position[a]	Distance from previous SNP	Minor allele	MAF[b] observed	MAF from NCBI*	SNP location relative to *HSD17B1*
rs2830	37958089		C	0.485	.392	Exon1
rs2676530	37959481	1392	A	0.230	.263	Intron 4
rs676387	37959799	318	T	0.259	.337	Intron 4
rs605059	37960432	633	T	0.482	.443	Exon 6
rs598126	37970046	9614	T	0.491	.429	Exon 4 of COASY

[a] Physical position on chromosome: Hg18, March 2006 assembly, dbSNP build 130.
[b] MAF: Minor allele frequency.
*http://www.ncbi.nlm.nih.gov/.

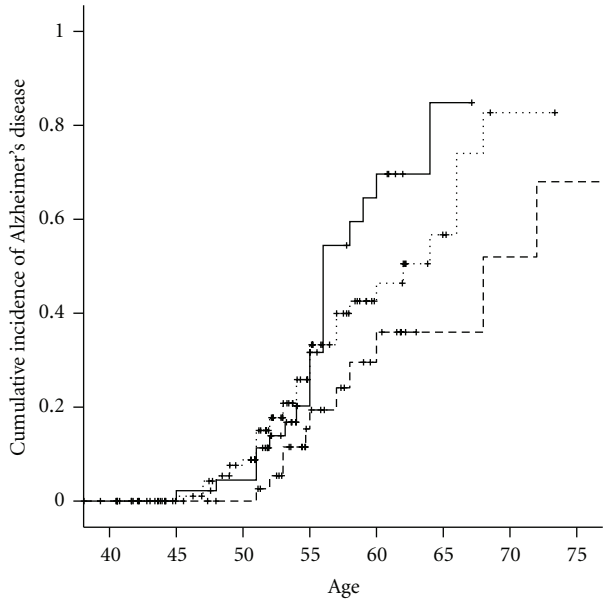

Figure 1: Cumulative incidence of Alzheimer's disease by *HSD17B1* rs605059 genotype in women with Down syndrome. TT - - - -. CT. . . . . CC—.

Table 3: Alzheimer's disease risk by HSD17B1 genotype in women with Down syndrome.

HSD17B1 genotype*	*N*	AD	HR (95% CI)**
rs2830			
CC	49	15 (30.6)	0.7 (0.3.5)
CT	101	31 (33.7)	0.9 (0.5–1.8)
TT	56	15 (26.8)	1.0 (reference)
rs2676530			
AA	14	7 (50.0)	1.5 (0.7–3.3)
AG	75	17 (22.7)	0.7 (0.4–1.2)
GG	135	47 (34.8)	1.0 (reference)
rs676387			
TT	22	9 (40.9)	**2.7 (1.2–5.8)**
GT	72	23 (31.9)	1.4 (0.8–2.4)
GG	129	40 (31.0)	1.0 (reference)
rs605059			
CC	59	20 (33.9)	**3.0 (1.4–6.8)**
CT	107	34 (31.8)	2.0 (0.98–4.2)
TT	51	12 (23.5)	1.0 (reference)
rs598126			
CC	58	15 (34.)	**2.2 (1.1–4.4)**
CT	119	37 (31.1)	1.4 (0.7–2.6)
TT	54	20 (27.8)	1.0 (reference)

** Hazard ratio for AD, adjusted for age, ethnicity, level of intellectual disability, BMI, and the presence of an *APOE* ε4 allele.
*Numbers vary because not all participants were genotyped for all SNPs.

cumulative incidence of AD over followup (Figure 1). The effects of carrying risk alleles for rs605059 were primarily seen in women over 60 years of age (Figure 1).

The relation of rs676387 and rs598126 to increased risk for AD was seen only among women homozygous for the risk allele (Table 3) and was associated to a two- and one half-fold hazard ratio ($HR_{rs676387}$ = 2.7, 95% CI: 1.2–5.8 and $HR_{rs5998126}$ = 2.2, 95% CI: 1.1–4.4) and with earlier onset but not higher cumulative incidence of AD (Figures 2(a) and 2(b)).

3.3. Haplotype Analysis of the Three SNPs in a Cox Proportional Hazards Modeling. We first computed the most likely haplotypes for each individual and then used the haplotypes as a "super locus" to estimate hazard ratios controlling for potential confounders. Our haplotype analysis using rs676387-rs605059-rs598126 revealed that the carriers of haplotype TCC had earlier onset of AD, after adjusting for the presence of an *APOE* ε4, allele level of intellectual disability, ethnicity, and BMI (hazard ratio = 1.8, 95% CI, 1.1–3.1).

4. Discussion

Three of the five SNPs examined in *HSD17B1* were associated with increased risk of AD. Women who were heterozygous or homozygous for the C allele at rs605059 were two to three times as likely to develop AD as those carrying the TT

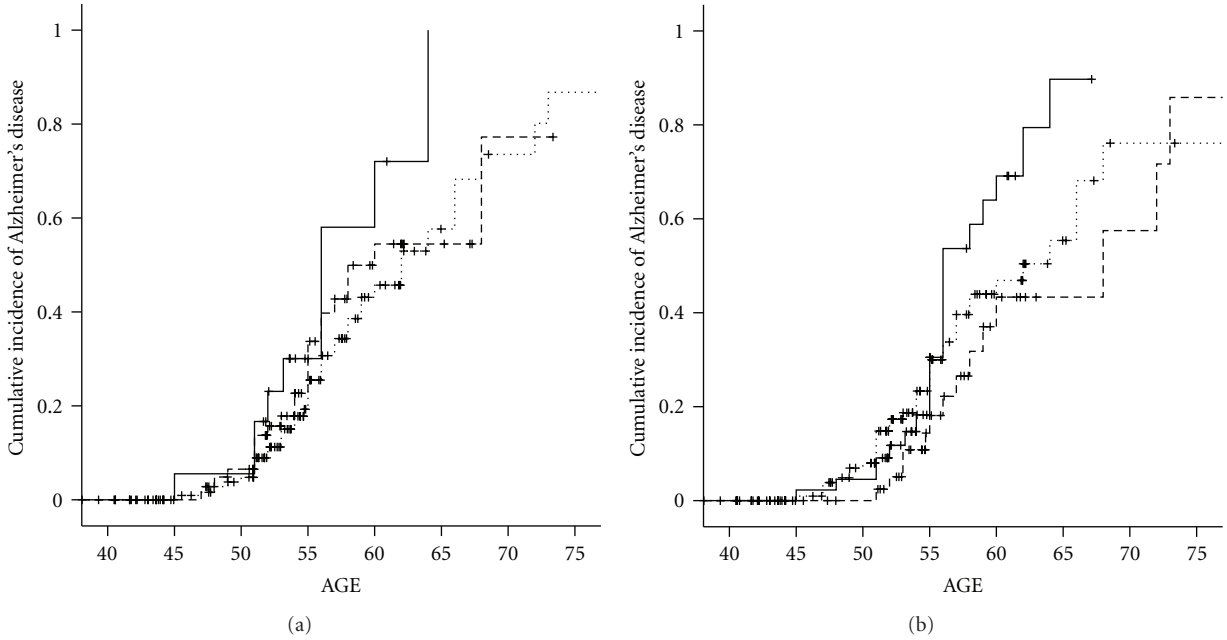

FIGURE 2: (a) Cumulative incidence of Alzheimer's disease by *HSD17B1* rs598126 genotype in women with Down syndrome. TT - - - -. CT. . . . . CC—. (b) Cumulative incidence of Alzheimer's disease by *HSD17B1* rs676387 genotype in women with Down syndrome. CC - - - -. CT. . . . . TT—.

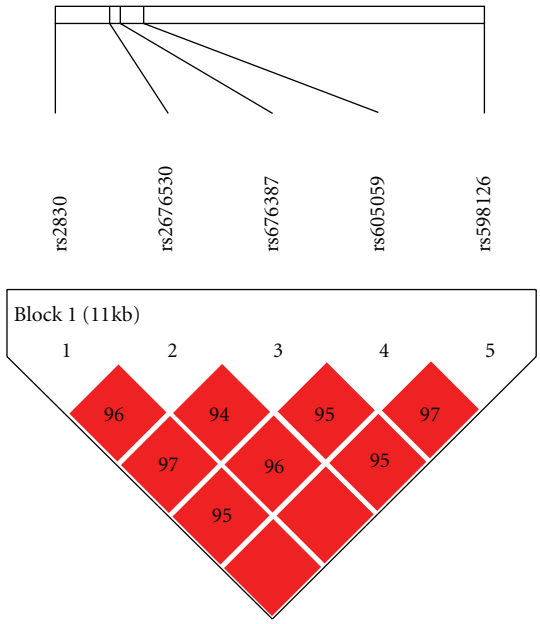

FIGURE 3: Linkage disequilibrium patterns for SNPs in *HSD17B1*.

genotype. Women with DS homozygous for the T allele at rs676387 or the C allele at rs598126 had 2.7 and 2.2-fold increased risk of AD, respectively, compared with women without these risk alleles, although risk was only slightly increased in women who were heterozygous for the risk allele. Carrying a high-risk allele at rs605059 was associated with both early onset and higher cumulative incidence, while carrying a high-risk allele at rs676387 or at rs598126 was associated primarily with earlier onset. Haplotype-based Cox proportional hazards model continued to support that TCC carriers had an almost 2-fold risk of developing AD after adjusting for covariates.

Polymorphisms or haplotypes in *HSD17B1* have been associated with increased risk for estrogen receptor-negative breast cancer, endometriosis, and endometrial cancer, but these associations have been modest and inconsistent [13, 15–32]. The *HSD17B1* gene encodes the enzyme HSD1 which catalyzes the conversion of estrone to estradiol. One pathway by which variants in *HSD17B1* could influence risk for AD is through changing the activity of HSD1 leading to changes in circulating estrogen levels. After menopause, the primary form of estrogen is estrone, which is formed in adipose tissue, muscle, liver, bone marrow, brain, and fibroblasts from aromatization of circulating androstenedione [44]. Increased body mass index in postmenopausal women is correlated with higher levels of serum estradiol and estrone [45, 46]. Low BMI has been found to be a risk factor for cognitive decline and risk for AD in late life [39, 47–49], and BMI may decline decades before onset of AD [50]. Among postmenopausal women not using hormone replacement therapy, nonobese women (<25 BMI) who were heterozygous or homozygous for the C allele at rs605059 had lower levels of estradiol and a lower estradiol/estrone ratio than women carrying the TT genotype [13], while no corresponding effects on estrone or estradiol levels were seen in women with BMI > 25. Our results showing earlier age at onset and higher cumulative incidence of AD among women carrying the C allele at rs605059 are consistent with this finding. Among non-obese women, variants in *HSD17B1* have also been associated with a more rapid rate of decline in estradiol

levels during the perimenopausal period [51]. Low estrogen levels have been associated with increased risk of cognitive impairment and AD [38, 52–59], although some studies have found high levels of total estradiol in women with AD [60, 61]. A role for low estrogen in AD has also been supported by experiments in which estrogen deficiency accelerated amyloid plaque formation in transgenic mouse models of AD [62, 63]. The findings from this study are consistent with a role for *HSD17B1* in modifying risk of AD through influences on peripheral or central estrogen levels and point to the potential for hormonal replacement therapy to delay onset of AD in this high-risk population.

HSD17B1 belongs to the family of short-chain dehydrogenases/reductases (SDRs) of which at least 11 other 17-beta HSD types are under study, named for their sequence homology to *HSD17B1* [64]. One of these, 17-beta HSD10, has demonstrated involvement with AD through binding with amyloid-beta [65]. While the substrate activity of *HSD17B1* is quite restricted, unlike that of 17beta HSD10, the multifunctionality of all SDRs is just beginning to be explored. For example, increased expression of *HSD17B1* and aromatase have been found in the prefrontal cortex of AD patients during the later stages of the disease [33]. It has been suggested that estradiol is upregulated in astroglia during AD, much as it is in reactive astroglia following brain injury, and increased expression of aromatase and *HSD17B1* may determine differences in levels of protective neurosteroids in the prefrontal cortex [33]. Continued work on genetic factors affecting neurosteroid activity may help to understand differences in rates of cognitive aging and risk of dementia.

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Review Article

β-Secretases, Alzheimer's Disease, and Down Syndrome

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Individuals with Down Syndrome (DS), or trisomy 21, develop Alzheimer's disease (AD) pathology by approximately 40 years of age. Chromosome 21 harbors several genes implicated in AD, including the amyloid precursor protein and one homologue of the β-site APP cleaving enzyme, BACE2. Processing of the amyloid precursor protein by β-secretase (BACE) is the rate-limiting step in the production of the pathogenic Aβ peptide. Increased amounts of APP in the DS brain result in increased amounts of Aβ and extracellular plaque formation beginning early in life. BACE dysregulation potentially represents an overlapping biological mechanism with sporadic AD and a common therapeutic target. As the lifespan for those with DS continues to increase, age-related concerns such as obesity, depression, and AD are of growing concern. The ability to prevent or delay the progression of neurodegenerative diseases will promote healthy aging and improve quality of life for those with DS.

1. Introduction

According to the CDC, 1 in 700 infants born have Down syndrome (DS), approximately 400,000 people in the US and 6 million people world-wide. DS is caused by an extra copy of chromosome 21 that arises during gametogenesis. In 95% of cases, this occurs as the result of chromosomal nondisjunction [1]. This is usually due to improper segregation of chromosomes into daughter cells during meiosis I (Figure 1), although nondisjunction in meiosis II also occurs. This results in gametes that have two copies of chromosome 21 (HSA 21), and upon fusion with another gamete, results in trisomy 21. Although HSA 21 is the smallest human autosome, the chromosome encodes more than 400 known genes [2], a number that may increase with further study. Less frequently, DS occurs due to somatic mosaicism or translocations [1]. DS presents with an easily recognizable phenotype, including a characteristic set of facial features, delayed development, and varying levels of intellectual disability, shortened stature, muscle hypotonia, joint laxity, AD-like neuropathology, and a heterogeneous range of other traits.

Advances in health care have led to improved longevity for individuals with DS, with the expected lifespan now approaching 60 years. While advanced maternal age is the only well-documented risk factor for DS [3], many socioeconomic and environmental factors that are difficult to evaluate may affect prevalence and survivability. With aging, the DS population faces an entirely different set of challenges. By the late 1800s, it was documented that individuals with DS develop plaque and tangle neuropathology that is similar to the one described in 1906 by Alois Alzheimer and is now known as Alzheimer's disease (AD) pathology (reviewed in [4]). AD is a disease that has progressed in our social consciousness from a peculiar rarity less than half a century ago to one of the greatest public health concerns of our generation [5]. We now know that essentially all individuals with DS develop AD-like pathology by the fourth decade of life. Interestingly, this predated the finding that an extra copy of chromosome 21 causes DS by almost 50 years [6]. Clues as to how this predisposes individuals with DS to AD-like pathology became more clear with the finding that HSA 21 harbors the genes for the amyloid precursor protein

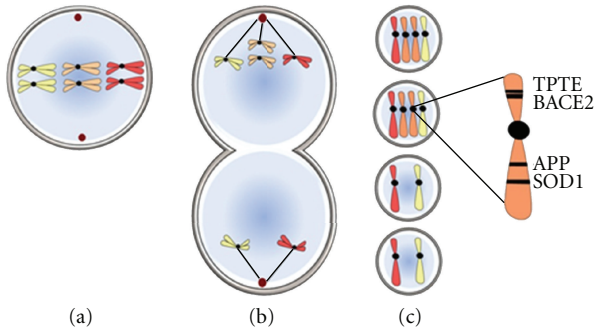

Figure 1: Chromosomal nondisjunction. (a) Most often Down syndrome (DS) occurs as an error in meiosis I (usually in the oocyte). Chromosomal nondisjunction, or improper segregation of chromosome 21 (the smallest autosome; orange), results in one precursor cell having 2 copies (b), upper half) while the other has zero (b), lower half). (c) Meiosis II then proceeds, with the outcome being two gametes that possess an extra copy of chromosome 21 which, after fusion with another gamete, bears 3 copies of chromosome 21; the genetic condition known as DS or trisomy 21. Also produced in this process are two nonviable gametes that possess zero copies of chromosome 21 (bottom).

(APP) and BACE2, two genes directly implicated in AD pathogenesis.

Alzheimer's disease is a devastating disease and is a growing public health concern as our population ages. The most common form of dementia among the elderly, AD is already taking a toll on our health care system, and many families struggle to provide necessary care. AD manifests as a progressive cognitive decline, including memory loss, speech dysfunction, and impaired spatial orientation, as well as a host of other symptoms [7]. In the general population, AD manifests in two forms: an autosomal dominant early onset form of the disease, familial AD (FAD), that accounts for less than 1% of disease cases, and the more common sporadic form of late-onset AD. Age of onset distinguishes the two groups, but clinical presentation and neuropathology are identical [8]. Thus, studying FAD gene mutations has provided insight into the molecular mechanisms that lead to neuropathology [9–12], even though the process may begin as much as 20 years before the patient begins to present clinically with symptoms [13].

2. The Molecular Neurobiology and Histopathology of AD

AD is characterized by the presence of two neuropathological lesions, extracellular plaques composed largely of a 40–42 amino acid peptide called β-amyloid (Aβ), and intracellular tangles and striated neuropil threads composed of a hyperphosphorylated form of the cytoskeletal protein tau [14–16]. Synapse loss in areas of the brain vital for learning and memory correlates with a patient's performance on cognitive tests even in cases of mild AD and precedes neuronal loss, which becomes prevalent in mild-AD. [17, 18]. This neuropathology eventually encompasses most of the brain, which ultimately becomes atrophied, with enlarged ventricles and significantly less overall brain weight than a comparatively aged healthy brain.

Characterization of genomic mutations present in early onset FAD led to the amyloid cascade hypothesis [19]. The amyloid precursor protein (APP) is a ubiquitously expressed type 1 transmembrane protein similar in structure to a receptor [20], but after years of intense study no universally accepted ligands have been identified [21]. The processing of the protein is now known in considerable detail [22–24] (Figure 2(a)). Nonamyloidogenic APP processing by α-secretase on the cell surface results in cleavage within the Aβ peptide fragment thereby abrogating Aβ peptide formation and resulting in secretion of a large fragment, sAPPα. The resultant transmembrane c-terminal fragment (CTFα) is a substrate for γ-secretase processing, but results in secretion of a peptide fragment much smaller than Aβ, called p3. Cleavage of APP by a transmembrane aspartyl protease, β-site APP site cleaving enzyme (BACE), occurs in the endocytic pathway (Figure 2(b)) and results in the transmembrane fragment CTFβ. Subsequent cleavage in the transmembrane domain of CTFβ by γ-secretase generates secreted Aβ peptide fragments 38–43 residues in length. Cleavage of either CTFα or CTFβ by γ-secretase also results in the generation of a small, cytosolic fragment (AICD) of poorly understood function. FAD-linked mutations in APP generally result in an increase in Aβ_{42} production [25, 26]; this is thought to be the most toxic peptide species generated by this noncanonical APP processing pathway and leads to aggregation and formation of higher order structures including oligomers (reviewed in [27]) that damage neurons and induce pathogenesis [28]. This slightly longer peptide fragment is more hydrophobic and is thought to seed neuritic plaque deposition by causing aggregation of other species that are more soluble, such as Aβ_{40} [29, 30].

The 400 known genes on HSA 21 represent many protein families and diverse functions, including the transmembrane phosphatase with tensin homology (TPTE) and superoxide dismutase (SOD1). HSA 21 harbors at least two genes implicated in the development of AD-like pathology (Figure 1(c)). The first is APP, the substrate from which the pathogenic Aβ peptide is derived. The second is BACE2, an aspartyl protease with ~65% sequence homology to BACE1, the major form of β-secretase in the brain. BACE1 was originally discovered by multiple groups as the primary β-secretase responsible for Aβ generation in the brain [21, 31–34], and the homologue BACE2 was discovered shortly thereafter [35, 36]. The β-secretases belong to the pepsin family of aspartyl proteases and are the only transmembrane domain containing members. The BACE1 gene is found on chromosome 11 and encodes a 501 amino acid protein, while the BACE2 protein is found on chromosome 21 and encodes a 518 amino acid protein (reviewed in [37]). Like other aspartyl proteases, both BACE1 and BACE2 have an N-terminal prodomain that is cleaved by a furin-like protease or through autoproteolytic cleavage [38] to generate the mature enzyme. One of the primary differences between the enzymes occurs within the C-terminal portion of the proteins, with the BACE1 active-site containing 3 disulfide bonds, while BACE2 has 2 [39].

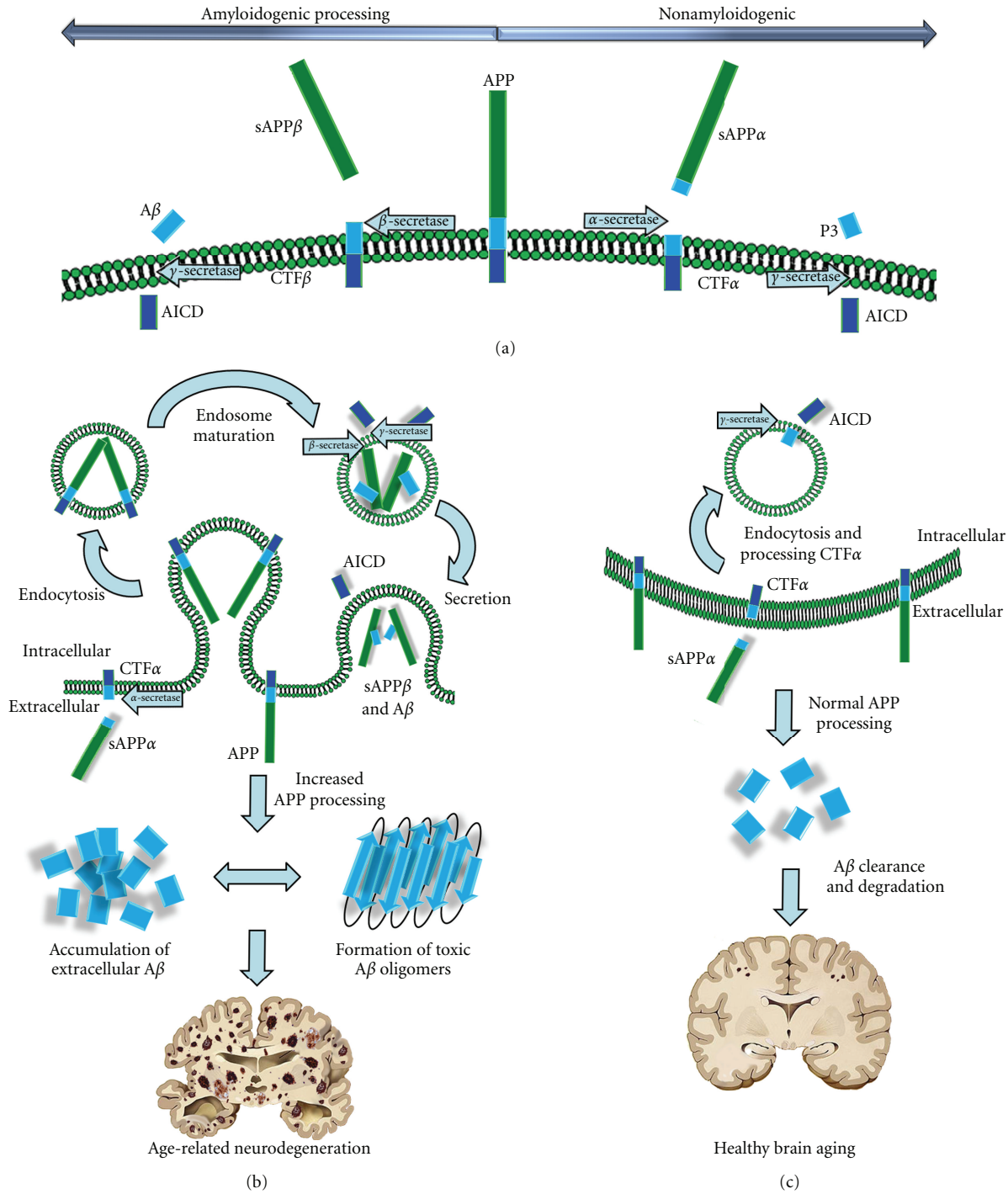

Figure 2: APP processing and imbalance in age-related neurodegeneration. (a) The amyloid precursor protein is processed either by an amyloidogenic pathway (left) or a canonical pathway (right). Canonical processing by α-secretase results in secretion of a large extracellular fragment, sAPPα. Importantly, this cleavage occurs within the Aβ peptide fragment (light blue), preventing its formation. A membrane bound C-terminal fragment, CTFα, then becomes a substrate for γ-secretase. This cleavage occurs within the membrane, releasing a short extracellular p3 peptide, and the APP intracellular domain (AICD, dark blue). Amyloidogenic processing occurs as APP interacts with β-secretase, or BACE, in the endocytic pathway. This generates the secreted sAPPβ, and a longer C-terminal fragment, CTFβ; γ-secretase cleavage of this fragment generates Aβ and AICD. (b) In Down syndrome, the overexpression of APP on the cellular surface results in increased amounts of APP being endocytosed. In mature endosomes, BACE (an enzyme that is more active at acidic pH) then cleaves APP resulting in increased amounts of CTFβ and Aβ peptide (light blue) being secreted outside the cell. Increased extracellular accumulation of toxic Aβ species, particularly Aβ_{42}, results in the formation of Aβ oligomers. These oligomers then overwhelm the brains capacity for clearance and degradation and form extracellular plaques, ultimately leading to neurodegeneration and severe brain atrophy. (c) Normally, most APP is cleaved by the α-secretase, secreting sAPPα. CTFα is endocytosed and then processed by γ-secretase, resulting in formation of the p3 peptide, which is secreted, and releasing the AICD into the cytosol. BACE processing of APP does occur to generate Aβ (blue), but these are degraded and cleared. While few small plaques may accumulate with aging, they are much smaller and fewer in number than those associated with disease.

3. β-Secretases and Neuropathology

Since its discovery little more than a decade ago, a vast body of work has amassed supporting the role of BACE1 in AD. BACE1 activity has been established as the rate-limiting step in formation of the Aβ-peptide. BACE1 levels increase slightly during the normal aging process [40, 41], but it is well established that both BACE1 protein and enzymatic activity are further increased in the AD brain [42–44]. In the Swedish familial form of AD, an APP mutation at the β-site makes the protein a more efficient substrate for BACE, resulting in early onset dementia and a more rapid disease progression [45]. Importantly, BACE1 knockout prevents formation of the Aβ peptide in vivo, a finding that solidly supports BACE1 as the major β-secretase in the brain, and a prime therapeutic target for AD [46]. Although phenotypic changes in BACE1 knockout mice are subtle, it is likely that BACE1 is involved in myelination [47, 48] and is important during development and following traumatic brain injury [48, 49]. Unlike in AD, BACE1 activity in DS does not appear to be significantly increased [50]. While some reports indicate a trend toward an increase, the absence of a robust effect likely indicates that the overexpressed APP is more important for driving AD-like pathology in DS than an increase in enzymatic activity, [50–52] although other cellular processes may be involved [53].

Because BACE2 is located on chromosome 21 and initial reports indicated an ability to generate the Aβ peptide from APP [54], it seemed plausible that this enzymatic activity might contribute to AD pathology in DS [35]. Recent evidence indicates that BACE1 and BACE2 activities and expression are highly correlated in the brain, including in individuals with DS [50]. However, significant effort from multiple groups has uncovered little evidence to support a role for BACE2 in driving the disease process. While BACE2 mRNA is increased in DS [55], posttranscriptional regulatory mechanisms either prevent an increase in translation or affect flux of the protein by increasing the rate of degradation. Many groups have reported that levels of BACE2 protein in the DS brain are comparable to control brains in various brain regions [50, 55–57]. Even though structural studies indicate that the active sites of both BACE1 and BACE2 are very similar [39], overexpression studies of BACE2 in both primary and immortalized cell culture models generally result in decreased Aβ production [58]. Other studies indicate that BACE2 has a higher propensity to cleave APP downstream from the BACE1 protease site, actually abrogating Aβ formation [37, 58, 59]. In vivo studies using transgenic mice that overexpress BACE2 alone [60] or cooverexpress both BACE2 and APP [61] do not show a resultant increase in Aβ peptide in the brain. These findings taken together indicate that BACE2 is probably not responsible for AD pathology in the DS brain and, indeed, may have a protective function.

4. APP and Aβ

There is much debate about which characteristics confer toxicity to the Aβ peptide. The N-terminal end of the peptide, formed by β-secretase cleavage, is fairly heterogeneous and subject to various modifications. The C-terminus, produced by intramembrane processing of the CTF by the γ-secretase, yields a peptide 39–43 amino acids long, with Aβ_{40} and Aβ_{42} being the most abundant species. The peptide likely exists as a dynamic pool of forms ranging from soluble dimers through higher order oligomers that become increasingly insoluble with size and result in plaque deposition. While many of the events regarding this process are poorly understood, it is likely driven biochemically by sequestration of hydrophobic regions from the aqueous environment [62]. It is widely accepted that the 42 amino acid peptide is more hydrophobic and aggregate prone and is proposed to seed plaque formation in the brain. Aβ_{42} is the first peptide species to form extracellular deposits in the DS brain, and these deposits are abundant in brains from young individuals with DS by 12 years of age, approximately 20 years before significant Aβ_{40} and tau histopathology can be found [63].

The Aβ peptide is a fragment of APP, a transmembrane protein of unknown function. Recently, it was proposed that APP stimulates neuroprogenitor cells to develop into various glial cell lineages and could be a possible contributor to the decreased neurogenesis and delayed development seen in DS [64]. A role in the vasodilation process has also been suggested and represents a potential mechanism for APP-mediated cerebral amyloid angiopathy, a process that could contribute to early neuropathology in AD [65]. The APP gene is found in the DS obligate region, and the protein is overexpressed in the adult DS brain [50, 56]. Overexpression of APP leads to dysfunction of the endocytic system, resulting in increased turnover from the cellular surface, thereby increasing the likelihood that APP will encounter β-secretase and be processed via the amyloidogenic pathway [66]. This will result in more intracellular APP carboxyl-terminal fragment(s) cleaved at β-site(s) (CTFβ), and in turn more Aβ will be generated in the DS brain. Given that β-secretase itself does not appear to specifically increase in DS [50], it would thus appear that APP overexpression is the main driver of AD-like pathology in the brains of elderly DS individuals.

5. Conclusion

While there are similar neuropathological changes in people with DS compared to AD, the brains of these populations are quite different. The DS brain is slower to develop and smaller at maturity than the brain of a diploid individual, weighing less than 1250 and often under 1000 grams, several hundred grams less than normal. Anatomically, the DS brain is more rounded with a distinct fore-shortened shape, and smaller frontal lobes, hippocampi, and cerebellum (reviewed in [4]). The brain in older individuals with DS is susceptible to cell loss in both cortical and subcortical regions, resulting in dysfunctions in both neurotransmitter systems and neuronal circuitry.

Emerging evidence from both fetal and adult DS tissues and animal models of DS indicates that changes at the molecular level are more wide spread than previously acknowledged. While there are about 400 known genes on chromosome 21, a meta-analysis of the transcriptome and

proteome reveals that many more are affected. Several—but not all—genes on chromosome 21 were overexpressed, while expression of others was unchanged or even decreased [67]. This indicates that the in vivo state is the result of a more complex interplay of factors than a simple gene dosage effect. There may be over 300 genes that are significantly changed in DS, the majority of which are not located on chromosome 21, and many of which have known roles in early developmental processes. The role of these various changes in development and the penetrance of many of the typical phenotypes of DS is largely unknown. Recently exon tiling arrays have been used to interrogate the role of various genomic loci in DS features, using rare segmental trisomies [68]. Importantly, this work highlights that the obligate region of chromosome 21 is more heterogenous than anticipated and may not exist at all, as individuals with segmental trisomies can still present with a moderate to severe DS phenotype. One of the patients characterized, a 65-year old without an additional copy of APP, did not have dementia or indication of amyloid accumulation when assessed by brain imaging, supporting a causative role for APP overexpression in neuropathology in DS [68].

In the general population, a definitive neuropathological diagnosis of AD requires that the classical hallmarks of AD, namely, neuritic plaques and neurofibrillary tangles, to be present along with a clinical history of dementia. Although this characteristic AD-like pathology is present by the fourth decade of life, not all individuals with DS develop dementia, even with complete trisomy 21 [69]. Even though changes in cognitive ability and social withdrawal are often reported by caregivers of middle-aged persons with DS, there is some controversy about whether this represents a clinically defined dementia [4]. Prevalence rates for dementia in DS vary considerably between studies, but are approximately 15%, slightly higher than that in the general population; however, in DS, the dementia occurs at significantly younger ages (reviewed in [70]). Cognitive testing for DS has proven difficult, which is not surprising given the wide range of intellectual disabilities presented. Also, because there is often little cognitive data for individual patients before their decline, establishing a cognitive baseline is not often possible for individuals. These issues at the individual level make it difficult to elucidate effects in groups, resulting in floor effects plaguing cognitive tests, and difficulty making conclusions regarding population-wide affects in DS [71, 72]. A better understanding of the cognitive strengths and weaknesses of individuals with DS (reviewed in [73]) and how these change over time represents a huge need for the DS community. Recently, much effort has been put into developing cognitive tests specifically for DS, such as the Arizona Cognitive Test Battery [74]. These testing methods that can be used across a wide range of ages and cultures with little dependence on language skill are an important step forward. In addition, both functional and cognitive abilities are assessed, which are particularly useful for longitudinal studies of basic cognitive ability in persons with DS and discerning if they do indeed develop AD. As a diagnosis of AD requires both neuropathology and dementia, it is important for many reasons that we know the clinical consequences of AD-like pathology in DS versus the non-DS population.

DS is commonly recognized as a model for AD pathology, and is very much proof of principle for the amyloid cascade hypothesis, because the additional copy of APP in DS results in pathology long before it occurs in the general population. As such, if the progression to dementia is delayed or absent in DS, this may help us elucidate a therapeutic strategy that may be applicable to patients with familial or sporadic AD as well. Therapies to treat Alzheimer's disease in both the DS population and general population are limited. No pharmacological agents have been described that are able to alter disease progression. Symptoms may be improved by a cholinesterase inhibitor (donepezil, rivastigmine, galantamine), or NMDA receptor antagonist (memantine) (reviewed in [75]). Current goals include determining which biomarkers are indicative of the disease process years before development of pathology, which may lead to therapeutics designed to alter the disease process. Still, many questions remain. Although the pathway driving the degenerative process in DS may be different than the one in familial or sporadic AD, and is likely fueled by substrate (APP) overexpression, the neuropathological hallmarks of the disease are the same. How much do these pathways overlap compared to sporadic AD that occurs in the general population? Are there factors responsible for controlling progress for dementia that are altered in DS, and are these a direct or indirect consequence of an extra copy of HSA 21? Many non-DS individuals who have been followed longitudinally and come to autopsy have sufficient neuritic plaques and neurofibrillary tangles to meet the critera for a neuropathological diagnosis of AD, yet there is no evidence to suggest they experienced cognitive impairment or decline, and so are referred to as preclinical AD [76]. Although it is possible that they would eventually progress to dementia, it is also possible that these individuals exhibit a compensatory mechanism that allows them to endure this neuropathology relatively unscathed. A similar mechanism may be at work in DS.

While there is much to learn, developing and executing longitudinal studies for persons with DS is difficult, and success will depend on an integrated, informed, and motivated network of parents and caregivers of persons with DS, medical professionals that better understand the range of primary and secondary complications that result from DS, and involvement and outreach from the research community. This process has already begun as two goals stemming from the National Institutes of Health's Research Plan on Down syndrome will be realized within the next year. The first is the development and testing of a national registry for DS, and the second is the establishment of a consortium to bring clinicians and researchers together [77]. These are exciting steps for the DS community and hopefully just the beginning of many resources that will benefit individuals with DS. However, there are still many challenges and areas where improvements are needed, including identifying socioeconomic factors that impact the early development and increased risk of mortality among certain ethnicities; developing learning tools and programs specifically for intellectual disabilities; educating families and healthcare

personnel so individualized health plans and testing for routine and secondary afflictions can be monitored routinely; performing routine functional and cognitive testing prior to decline; and finally, using therapeutics for age-related concerns such as depression and AD.

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Review Article

The Use of Mouse Models for Understanding the Biology of Down Syndrome and Aging

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Down syndrome is a complex condition caused by trisomy of human chromosome 21. The biology of aging may be different in individuals with Down syndrome; this is not well understood in any organism. Because of its complexity, many aspects of Down syndrome must be studied either in humans or in animal models. Studies in humans are essential but are limited for ethical and practical reasons. Fortunately, genetically altered mice can serve as extremely useful models of Down syndrome, and progress in their production and analysis has been remarkable. Here, we describe various mouse models that have been used to study Down syndrome. We focus on segmental trisomies of mouse chromosome regions syntenic to human chromosome 21, mice in which individual genes have been introduced, or mice in which genes have been silenced by targeted mutagenesis. We selected a limited number of genes for which considerable evidence links them to aspects of Down syndrome, and about which much is known regarding their function. We focused on genes important for brain and cognitive function, and for the altered cancer spectrum seen in individuals with Down syndrome. We conclude with observations on the usefulness of mouse models and speculation on future directions.

1. Why Use Mouse Models?

Down syndrome (DS) is diagnosed by chromosome analysis, either prenatally (usually because of identified risk factors), or postnatally (typically because of the appearance of the infant). The DS phenotype is complicated and variable, thus models of DS must be able to address this complexity and variability.

Intellectual disability may be the most well-known feature of DS, but it is accompanied by behavioral, psychiatric, and neurological problems. In early infancy, people with DS function in the range of low typical development, but the intelligence quotient decreases in the first ten years of life, reaching a plateau in adolescence that extends into adulthood. Learning is complicated by a tendency to avoid cognitive challenges, and by a deficiency in language production. About 17.6% of individuals with DS less than 20 years of age have a psychiatric disorder, most often a disruptive behavioral disorder, such as attention deficit hyperactivity disorder, conduct/oppositional disorder, or aggressive behavior. About 25.6% of adults have a psychiatric disorder, most frequently depression or aggressive behavior. People with DS have a higher incidence of autism. By the fifth decade of life, neuropathological changes typical of Alzheimer's disease (AD) usually develop. Clinical signs and symptoms of AD are seen in 75% of people over 60 years of age. These are usually seizures, changes in personality, focal neurological signs, apathy, and loss of conversational skills [1].

The complexity of DS extends well beyond mental and neurological issues. For example, about half of people with DS are born with congenital heart disease, and heart disease can develop (or be initially identified) later in life. Adolescents and young adults with no known intracardiac disease can develop mitral valve prolapse and aortic regurgitation. People with DS are more likely to have

hematological disorders. These include polycythaemia in newborns, macrocytosis, transient myeloproliferative disorder, acute myeloid leukemia, and acute lymphoblastic leukemia. Between 38% and 78% of people with DS have conductive and/or sensorineural hearing loss. About 38% of children less than 12 months of age, and 80% age 5 to 12, have ophthalmological disorders requiring monitoring and intervention. The most frequent disorders are refractive errors, strabismus, and nystagmus. Resting metabolic rates are reduced in individuals with Downs syndrome, which results in a higher frequency of obesity, and children at ages 3 to 4 are more likely to be obese than not. Monitoring intake of calcium and vitamin D is important, since individuals with DS exhibit lower bone density. People with DS have a higher incidence of coeliac disease and hypothyroidism. Many disorders, such as arthritis, atlantoaxial subluxation, diabetes mellitus, leukemia, obstructive sleep apnea, and seizures, occur more frequently among individuals with DS than in the general population [1].

Given the complexity of the DS phenotype, computer models, *in vitro* models, models based on lower organisms, and so forth, are woefully inadequate for representing DS. Mouse models have many characteristics that make them well suited to the study of DS. First, mice are a higher organism with the requisite biological characteristics. Neurological, behavioral, cardiac, hematological, skeletal disorders, and so forth, can be studied using mouse models. Second, they are very well characterized. Mouse models have been extensively used in research, and a great deal is known about them. Additionally, they are commonly used in development and testing of drugs for treatment of various disorders, including ones associated with DS. Third, there are numerous practical issues that make mouse models especially attractive. Mice are small, they have a relatively short generation time, they reproduce rapidly, are inexpensive to maintain and house, and are easy to handle.

2. What Defines a DS Mouse Model?

Various genetically altered mice have been proposed as mouse models of DS, and rapid progress is being made creating new models. An important point to bear in mind is that no mouse model will be a perfect model of DS. Even though mice have many similarities to humans, there are significant, and obvious, differences; therefore, some aspects of DS simply cannot be adequately modeled in mice. For example, it is clear that one can use mice to study aspects of learning and memory, but they cannot serve as a complete model for human intellect. Mice have their own sets of behaviors that have been selected for over evolutionary time, and some of these behaviors are not relevant to studies of humans, with or without DS.

There are clear biochemical and metabolic differences between mice and humans as well, even though basic biochemical pathways have been conserved. For example, in humans, the end product of purine metabolism is uric acid, which is an antioxidant that may be relevant to the oxidative stress associated with DS. Indeed, individuals with DS accumulate unusually high levels of uric acid in their blood [2], which may be important in aging, and in neurodegenerative diseases associated with aging and DS [3]. This may be due to trisomy of the GART gene, which encodes an enzyme that catalyzes 3 steps of *de novo* purine synthesis, or it may be due to abnormal processing of uric acid by the kidneys of individuals with DS, or perhaps some other unknown mechanism. Mice, on the other hand, metabolize uric acid to allantoin, which is much more soluble and easily excreted. Therefore, modeling alterations in purine metabolism in mice may be difficult unless this metabolic difference is taken into account. One approach would be to use mice lacking uricase that accumulate uric acid as the end product of purine metabolism [4].

Another example is that mice metabolize folic acid somewhat differently than humans. Folate levels are about 10 fold higher in murine versus human plasma [5]. Therefore, alterations in folate metabolism in mice may not have the same metabolic consequences as similar alterations in humans. Abnormalities in folate metabolism or polymorphisms in genes encoding enzymes of folate metabolism have been associated with DS in many studies, although the significance of these polymorphisms is still unclear [6]. Adding to this complexity, it may be that particular polymorphisms in individual steps of folate metabolism may function only in the context of other polymorphisms, and that various suites of polymorphisms may have similar effects, making it difficult to compare studies [7]. Recent evidence also demonstrates that alterations in folate metabolism may be associated with specific aspects of DS, such as congenital heart disease [8]. Folate metabolism may also be related to the biology of aging and age-related disorders [9, 10]. Importantly, several genes necessary for folate metabolism reside on HSA21, including cystathionine beta synthase (CBS) [11]. Mutations in CBS are clearly associated with intellectual disability and cardiovascular disease [12]. The reduced folate carrier (Slc19a1), which is important for trafficking of folates in mammals, is also located on HSA21 [13]. Mutations or polymorphisms in Slc19a1 are associated with sensitivity to methotrexate [14]. It has been hypothesized that the presence of 3 copies of Slc19a1 in persons with DS may be partly responsible for their unusual sensitivity to folate analogues [15]. In the mouse genome, CBS maps to Mmu17 and Slc19a1 to Mmu10 (regions syntenic to HSA21). Numerous mouse models with alterations in CBS or Slc19a1 have been produced, including mice in which the endogenous mouse gene has been inactivated and replaced by the equivalent human gene [16–18]. So far, it has been difficult to learn much about human DS from studying these mice. The fact that these genes are present on different mouse chromosomes complicates the production of appropriate mouse models that are relevant to human DS.

Although Mmu16, 17, and 10 contain essentially all the known genes on HSA21, they are not completely analogous to HSA21. Some of the genes may be species specific in both humans and mice, and the chromosome regions may have regulatory sequences or copy number variations that may not encode proteins but are nonetheless important for phenotypic development. Sturgeon and Gardiner [19] have

published an excellent comparison of the relevant mouse and human genetic regions (along with chimpanzee), and the interested reader is referred to that work for further details.

An additional consideration is that manipulation of the mouse genome may have unexpected consequences. As discussed below, some trisomy mouse models have recently been shown to have unexpected additional chromosomal alterations.

Nonetheless, several different types of mouse models have been extremely useful in investigating aspects of DS, and models are becoming more accurate and sophisticated. Therefore, analysis of various types of mouse models is likely to be increasingly important in unraveling specific aspects of DS, with different types of models having different roles.

It is important to carefully consider the usefulness of mouse models that clearly show phenotypes relevant to DS. Specifically, if a particular mouse model of DS has a phenotype(s) reminiscent of DS, is the model appropriate for investigating DS? As clinical trials based on studies of various mouse models become more common, this becomes an exceedingly important question (see Section 5).

Even mice that do not show a phenotype reminiscent of DS may be quite useful in understanding DS. For example, mice in which a particular HSA21 syntenic gene has been inactivated by targeted mutagenesis may be crucial for understanding the function of that gene, informing understanding of its role in DS. Large-scale projects to inactivate every mouse gene individually and to evaluate the phenotype of each of the knockout mice are underway and should be extremely helpful in understanding the role of these genes in the DS phenotype (http://www.mousephenotype.org/). Moreover, these mice are useful in manipulating gene copy number in mice that are trisomic for particular Mmu chromosomal regions syntenic to HSA21. Comparison of several different models can increase confidence that phenotypes reminiscent of DS relate to human DS in a meaningful way. This approach was suggested in the first description of the isolation of the Ts65Dn mouse model, discussed below [20].

In this review, we have selected a few illustrative examples of mouse models of DS from the large number that exist. We describe mice trisomic for various regions of HSA21 or the mouse chromosomal regions syntenic to HSA21, selected transgenic (Tg) mice, and selected mice in which specific genes have been inactivated (knockout, or KO, mice). Further, where possible, we describe combinations of these models. We have focused primarily on genes for which both Tg and KO mice exist, and where KO mice have been combined with trisomic mice to elucidate function by restoring disomy. In this way, we have attempted to describe the wide range of options available in utilizing mouse models to study DS. We have also chosen genes that appear to be functionally related, for which considerable information regarding function is known, and that appear to be related to important DS phenotypes. Thus, we focus on the APP, RCAN1, and SYNJ1 genes because these appear to be important for synaptic plasticity and/or function, and are likely to be related to the intellectual disabilities seen in individuals with DS. We have also chosen RUNX1, ETS2, and ERG, a group of genes that are relevant to cancer. These genes are likely to be related to the altered incidence of cancer seen in individuals with DS.

3. Types of DS Mouse Models

3.1. Chromosomal Trisomy Mice. Mice trisomic for chromosome regions syntenic to HSA21, and mice that carry regions of HSA21, may be more complete models of the DS phenotype, since they are trisomic for many genes trisomic in individuals with DS. On the other hand, trisomy of multiple genes makes interpretation of results more complex. Numerous trisomic or transchromosomal mice have been produced. With the advent of chromosome engineering approaches, it is now possible to produce mice trisomic for any chromosome region. Figure 1 shows a graphical representation of the chromosome regions present in these models, and a representation of HSA21 and the syntenic mouse chromosome regions.

3.1.1. Ts16. The first mouse model of DS was the Ts16 mouse, which is trisomic for essentially all of Mmu16, including the part of Mmu16 syntenic to HSA21. These mice were produced by Alfred Gropp using a mouse breeding scheme that is selected for mice with centric fusions [21]. Several investigators, notably Dr. Charles Epstein, noted that some features of these mice were reminiscent of DS and hypothesized that these mice might present a model for certain aspects of DS [22]. This hypothesis was supported when several genes known to be located on HSA21, including SOD1, IFNAR, and GART, were found to be on Mmu16 [23–25]. This was the first evidence that mouse models of DS could be created.

Unfortunately, the Ts16 mice generally die during fetal development or very shortly after birth. Thus, they have proven useful for the study of embryonic/fetal development but are not very helpful for studies of aspects of DS during the lifespan, and certainly not for aspects of DS relevant to aging. However, cell lines derived from Ts16 mice have been used to study biological processes related to DS and potentially relevant to aging and age-related disorders [26, 27]. An important caveat is that the Ts16 mice are trisomic for many Mmu16 genes that are not located on HSA21.

3.1.2. Ts65Dn and Derivatives. The production by Muriel Davisson and colleagues of a mouse trisomic for only part of Mmu16, now known as the Ts65Dn mouse, was a seminal achievement in DS research [20]. They produced these mice by irradiating the testes of male mice, breeding them, and screening offspring for chromosomal rearrangements involving Mmu16. The Ts65Dn mouse is trisomic for roughly 94 genes syntenic to well-curated HSA21 genes, although this number is subject to change as analysis of the complete human and mouse genomic sequences continues [28]. It is the most well characterized and widely studied mouse model of DS. It is important to note that the Ts65Dn mouse is disomic for about 16 HSA21/Mmu16 genes [29], and it has recently been shown that these mice are also trisomic for a centromere proximal region of Mmu17. The chromosomal

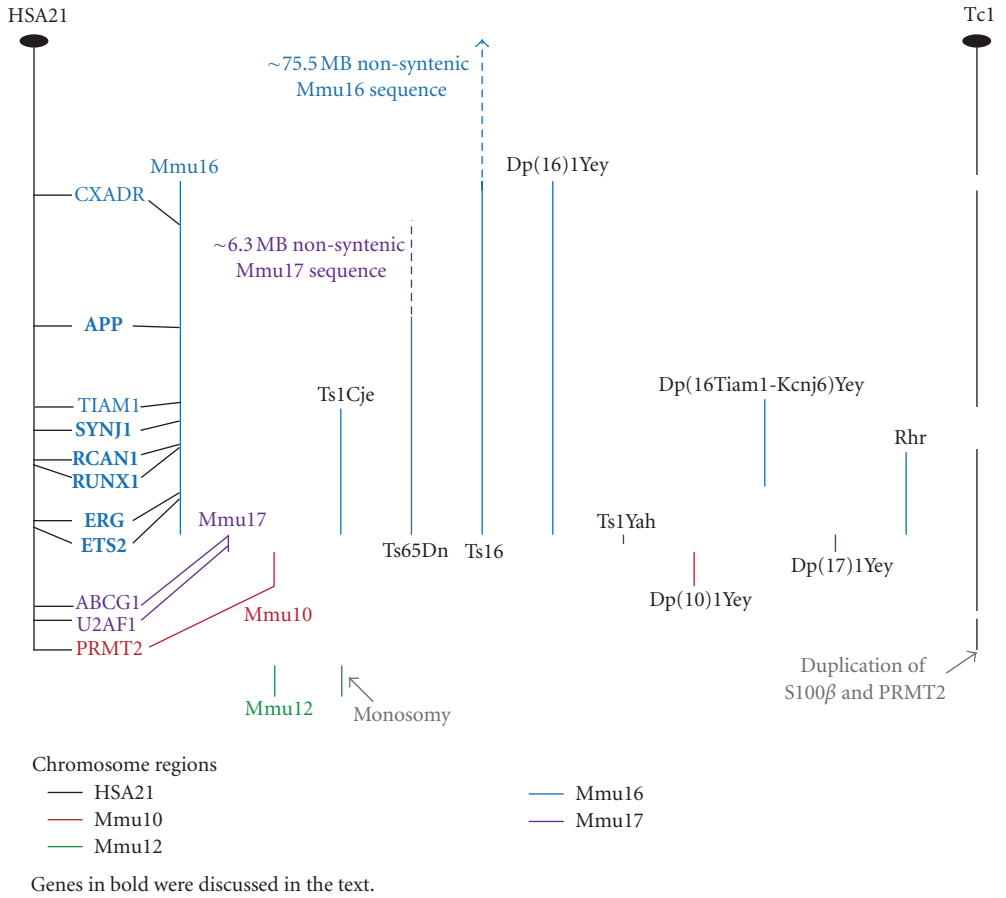

Figure 1: A graphical representation of HSA21 and the syntenic mouse chromosome regions from Mmu10, Mmu16, and Mmu17. The trisomic (or monosomic) chromosome regions present in 10 of the segmental mouse trisomies are also shown, with color-coding indicating the chromosome source of the region (see the key in the figure). The location of 11 HSA21 genes is shown, as well as their location on the syntenic chromosome regions, with text color indicating which syntenic chromosome. The dark ovals indicate the HSA21 centromere.

rearrangement site between Mmu16 and Mmu17 has been precisely defined [30, 31], and it turns out that Ts65Dn mice are trisomic for up to 60 Mmu17 genes, many of which are overexpressed in heart. Notably, two Mmu17 genes trisomic in Ts65Dn mice include Synj2 and Tiam2, which are related to the HSA21/Mmu16 encoded genes Synj1 and Tiam1. The phenotype of the Ts65Dn mouse is quite similar to the phenotype of mice trisomic for the entire region of Mmu16 syntenic to HSA21, and indeed to mice trisomic for all mouse chromosome regions present on HSA21 (discussed in more detail below). One difference between Ts65Dn mice and these models is that the trisomic region in Ts65Dn is present as a freely segregating extra chromosome, while in other models, the relevant chromosome region has been duplicated by chromosome engineering methods or in two cases (Ts1Cje and Ts2Cje) serendipitously. It has been argued that the presence of an extra chromosome in the Ts65Dn mice may make them a more acceptable model of DS. This proposal needs to be considered in light of observations on humans with DS due to translocations (i.e., not a freely segregating extra chromosome 21). The vast majority of these are Robertsonian translocations involving centromeric fusions [32]. Also, there are several examples of apparently balanced translocations between HSA21 and other human chromosomes that do not involve centromeres, resulting in an apparently classical DS phenotype [33–35]. Therefore, at least for phenotypes not related to reproduction, it seems that in humans the presence of an extra chromosome is not necessary for DS.

Ts65Dn mice have many features reminiscent of those seen in people with DS. These include anatomical features such as small brain regions (notably the hippocampus and cerebellum) and abnormal skull shape [36]. Other similarities include congenital heart defects [37], myeloproliferative disorders [38], decreased bone density [39], and altered incidence and response to certain cancers [40, 41]. Most notably, Ts65Dn mice exhibit deficits in learning, memory and behavior, as well as aspects of early neurodegeneration that may be relevant to early features of AD as well as aging [42, 43].

Ts65Dn mice show signs of what might be called premature aging and neurodegeneration. They show early loss of basal forebrain cholinergic neurons that may be related to loss of learning and memory ability in these

mice [42, 43]. Although the mice are trisomic for APP, they do not develop plaques or tangles characteristic of AD in humans or transgenic mouse models of this disorder. However, they do show increased expression of the APP gene and increased levels of the products of APP protein metabolism with age [44]. Moreover, old Ts65Dn mice accumulate tau/reelin containing clusters in the CA1 region of the hippocampus and extracellular tau/reelin granular deposits [45]. Similar deposits have been observed in mouse models of AD. Systemic aging has been examined in Ts65Dn mice, and there are indications that certain aspects of aging may be accelerated, for example, increased risk of lymphoma [46].

A number of treatments apparently improve learning and memory in Ts65Dn or prevent their decline [28]. Some of these are of particular relevance to the possibility of premature aging in these mice. For example, memantine, a drug used in humans to treat AD, appears to improve learning and memory in Ts65Dn [47–49]. Treatment of young (4 month old) Ts65Dn mice with a gamma secretase inhibitor has been reported to rescue learning and memory, potentially implicating the amyloid precursor protein (APP) or its metabolites in learning and memory deficits, even at an early age [50]. At least two laboratories have demonstrated that treatment with vitamin E can ameliorate learning and memory decline with age and concomitantly reduce signs of oxidative stress in the brains of Ts65Dn mice [51, 52]. One study suggests that vitamin E treatment is most useful if given perinatally and throughout life [52]. These findings are particularly relevant because numerous studies on the possible beneficial effects of vitamin E treatment on humans with AD and on humans with DS have been published or are underway. Although an early study appeared to show that large doses of vitamin E (2000 IU/day) slowed the loss of activities of daily living of persons with AD by about 11 months, other studies have not shown an effect, and the statistical significance of the initial study has been questioned [53–55]. A recent publication provides an interesting discussion of why human trials of vitamin E fail [56]. One obvious issue is that studies in which treatment has started after disease onset may fail because irreversible damage has already been done. This is consistent with studies suggesting that lifelong vitamin E supplementation of Ts65Dn mice may be most effective.

A recent study also indicates that choline supplementation during pregnancy and lactation improves aspects of learning and memory and emotion regulation in adult Ts65Dn offspring [57]. This is consistent with earlier reports that prenatal choline supplementation improves the learning and memory of diploid rats, well into adulthood [58, 59]. More recent studies show that perinatal choline supplementation has beneficial effects on the development of the hippocampus in mice as well [60, 61]. These studies did not assess the long-term effects of perinatal choline supplementation on mouse behavior or learning and memory. This effect may be related to the cholinergic deficits seen in Ts65Dn mice.

Several other treatments, including fluoxetine, PTZ, prodrugs for norepinephrine, xamoterol, and perhaps lithium and voluntary exercise, improve certain aspects of the Ts65Dn phenotype.

There have been a number of models derived from Ts65Dn mice. Some of these overcome specific weaknesses in the Ts65Dn model. For example, Ts65Dn mice carry a gene for retinal degeneration, which means some mice are blind and cannot be used for tests requiring vision. The gene has now been bred out of the Ts65Dn mice, and these mice appear otherwise to be essentially equivalent to the original Ts65Dn [62]. A second weakness of the Ts65Dn mouse is that males generally are functionally sterile. Ts2Cje mice are a Ts65Dn derivative in which the extra chromosome of the Ts65Dn mice has undergone a Robertsonian translocation with Mmu12 [63]. These mice appear to breed well, and males have increased, though still diminished, fertility. Recently, the Reeves laboratory has reported a method for breeding large numbers of Ts65Dn mice from Ts65Dn males [64].

3.1.3. Ts1Cje. The Ts1Cje mouse is trisomic for a shorter region of Mmu16 than the Ts65Dn mouse, containing roughly 75 genes syntenic to well-curated genes located on HSA21. This mouse is the consequence of an attempt to inactivate Sod1 by targeted mutagenesis, resulting in a translocation between Mmu16 and Mmu12. The Sod1 gene is inactivated in these mice. It has recently been shown that this mouse is monosomic for seven Mmu12 genes [30, 65]. Two of these, Abcb5 and Itgb8, are related to the HSA21 genes ABCG1 (located on Mmu17), and CD18 (located on Mmu10). Neither of these is trisomic in Ts65Dn or Ts1Cje.

The Ts1Cje mice are disomic for 19 genes that are trisomic in Ts65Dn (or Ts2Cje, which is apparently genetically equivalent, see Section 3.1.2) mice, including APP and SOD1. Therefore, a useful approach is to compare features associated with DS in the Ts2Cje and Ts1Cje mice. Presumably, differences between the two mouse strains are due to the difference in gene copy number, and common features are due to the common set of trisomic genes. An interesting comparison of Ts1Cje and the Ts2Cje mice indicates that both strains share enlarged brain ventricles and decreased neurogenesis [66]. On the other hand, learning deficits in Ts1Cje appear to be less severe than in Ts2Cje, and degeneration of basal forebrain cholinergic neurons is absent [67]. However, several neuroanatomical features related to DS, like regionally selective decrease in dendritic spines, are present in Ts1Cje but are less severe [68]. Given the recent recognition of the extent of trisomy of Mmu17 genes in Ts65Dn and the monosomy of Mmu12 genes in Ts1Cje, caution is necessary in interpreting the results of these comparisons. Moreover, as discussed above, it should be kept in mind that Ts65Dn mice are aneuploid and have a free extra chromosome, while Ts1Cje and Ts2Cje (and several of the mice described below) do not. The possibility exists that the presence of the extra chromosome in Ts65Dn mice may affect their phenotype [30].

An example of the usefulness of the Ts1Cje model has to do with the hypothesis that SOD1 and APP may be

important for oxidative stress, mitochondrial dysfunction, and tau hyperphosphorylation, perhaps associated with premature aging and neurodegeneration. It appears that all these features are observed in the Ts1Cje mouse model even though APP and SOD1 are functionally diploid in these mice [69]. Presumably other genes trisomic (or possibly monosomic) in Ts1Cje play a role in these abnormalities.

Rapamycin, an inhibitor of mammalian target of rapamycin (mTOR), has recently been shown to extend the health span and lifespan of diploid, noninbred mice [70, 71]. mTOR is a key regulator of metabolism and of dendritic morphology and synaptic plasticity. Interestingly, in Ts1Cje mice, levels of BDNF and phosphorylated Akt-mTOR are elevated. This results in abnormally high local dendritic protein translation, thought to play a key role in memory formation. Treatment of Ts1Cje neurons with rapamycin repairs this defect [72]. These findings suggest the possibility that rapamycin, or other inhibitors of mTOR, might be useful in treatment of learning and memory loss and intellectual disability in DS.

3.1.4. Ts1Rhr and Related Mice. Mouse models have the potential to contribute to the question of genotype-phenotype mapping in DS. Appropriately constructed mouse models should be useful for testing whether the postulated Down Syndrome Critical Region (DSCR, a small region of HSA21 critical for the development of DS) exists [73, 74]. Olson et al. [75] used chromosome engineering to produce the Ts1Rhr mouse, which is trisomic for the mouse equivalent of the hypothetical human DSCR. This region includes about 33 genes. In their initial report, it was shown that this mouse DSCR is not sufficient and, by examining mice monosomic for this region, largely unnecessary for the craniofacial phenotype seen in Ts65Dn mice and in people with DS. In later studies [76], it was shown that trisomy of the DSCR alone is necessary, but not sufficient, for the structural and learning and memory deficits (assessed by the Morris water maze) seen in Ts65Dn mice and in DS. However, a later, more comprehensive study utilizing behavioral tests considered more sensitive than the Morris water maze test revealed that the situation is considerably more complex than initially thought [77]. In this study, using the same mice, trisomy of this region was found sufficient to confer behavioral, neurophysiological, and synaptic phenotypes characteristic of DS. In all, 20 of 48 features related to DS were altered; however, some changes were less severe than in Ts65Dn (or Ts1Cje) mice. Moreover, the Ts1Rhr mice showed phenotypes that are not observed in Ts65Dn or other models, including increased body and brain weight and a larger posterior hippocampal region compared to diploid mice, which is not seen in DS and in Ts65Dn or Ts1Cje mice. The authors suggest that these findings may mean that people with partial trisomy 21 may have phenotypes not seen in full trisomy 21. As mentioned above, Ts65Dn mice have reduced bone density [39]. Ts1Rhr mice do not exhibit this phenotype, and mice monosomic for this region show decreased bone density [78]. These experiments illustrate the complexity of the DS phenotype and reinforce the concept that study of different mouse models is important for developing an understanding of how DS develops.

3.1.5. Ts1Yah. Ts1Yah mice are trisomic for the HSA21 syntenic region on Mmu17 between Abcg1 and U2af1, which contains 12 genes [79]. These mice have several interesting features. They have learning and memory deficits as measured by the open field, Y arm maze, and novel object recognition tests. However, they appear to learn more efficiently in the Morris water maze test than diploid mice. They also have larger and longer lasting long-term potentiation (LTP) responses than diploid control mice, probably related to their improved performance on the Morris water maze. This is a provocative finding that clearly supports the hypothesis that interaction of many regions of HSA21 is required for the DS phenotype. Indeed, as the authors point out, trisomy of certain genes or regions of HSA21 may actually improve some aspects of cognition. Such a compensatory mechanism has been hypothesized to exist in human DS as well [80].

Ts1Yah mice also illuminate some of the necessary precautions required when studying chromosomally engineered mice. Expression levels of the genes within the trisomic region (and in the companion monosomic mice) were measured. Expression of the two genes at the end of the triplicated or deleted region, ABCG1 and U2af1, was not altered in monosomic, disomic, or trisomic mice. Ubash3a, Tff2, Tff3, and Tmprss3 were expressed equally in monosomic and trisomic regions. The other genes in the region were expressed according to gene dosage. The Umodl1 gene, adjacent to the Abcg1 gene but not in the engineered region, showed apparent increased expression in the thalamus, but not in the hippocampus and cerebellum. These results demonstrate the importance of analyzing chromosomally engineered mice for unexpected effects on expression of genes near the rearranged chromosomal region.

3.1.6. Mice Trisomic (or Monosomic) for the Entire Mmu16, 17, and 10 Chromosomal Regions Syntenic to HSA21. It could be argued that the ideal mouse model of DS would be trisomic for all the mouse genes syntenic to HSA21 (i.e., the relevant parts of Mmu16, 17, and 10). A seminal achievement has been accomplished via a process involving production of mice trisomic for the relevant regions of each mouse chromosome using chromosomal engineering methods [29]. Then, mice trisomic for all three regions were produced via selective breeding. These mice have many features reminiscent of those seen in DS. Many of the abnormalities in learning, memory and hippocampus are very similar to those seen in the Ts65Dn mice.

The production of these mice allows examination of the effect of trisomy of each syntenic chromosome region individually [81], resulting in some intriguing findings. Dp(10)1Yey/+ mice, trisomic for the Mmu10 syntenic region, did not have any detected alterations in learning and memory behaviors or in hippocampal LTP. Trisomy of the syntenic region on Mmu17 in Dp(17)1Yey/+ mice resulted

in an increase in hippocampal LTP but no statistically significant change in learning and memory as assessed by the Morris water maze or contextual fear conditioning. The Dp(16)1Yey/+ mice (trisomic for the Mmu16 region syntenic to HSA21), on the other hand, showed abnormalities in hippocampal LTP and both the Morris water maze and contextual fear conditioning tests similar to those seen in the Ts65Dn mice. These results allow for some preliminary conclusions regarding the Ts65Dn mice and demonstrate the value of multiple mouse models. Specifically, since the Dp(16)1Yey/+ and the Ts65Dn mice have similar phenotypes, one could argue that the extra Mmu16 genes trisomic in the Dp(16)1Yey/+ mice do not contribute to these aspects of the Ts65Dn phenotype. Also, since the Dp(16)1Yey/+ mice are not trisomic for any of the Mmu17 genes trisomic in Ts65Dn, these genes are also unlikely to be important for the measured phenotypic changes. Finally, the minimal effect of the Mmu10 and 17 genes on the measured phenotypes suggests that these genes are not major contributors to the DS related phenotypes. However, these studies do provide evidence that the various chromosome regions may interact with each other, so care must be taken in making these conclusions. One must also keep in mind that variations in the tests used may lead to different interpretations regarding the relationship of the phenotype of these mice to DS. Also, more complete characterization of these mice may yet reveal that trisomy of the Mmu17 or 10 regions does lead to phenotypic alterations relevant to DS.

One important phenotype of DS is congenital heart defects. Considerable effort has been spent attempting to correlate partial trisomies of HSA21 with this phenotype [73, 82]. Analysis of the mice described above indicates that only the Mmu16 region is required to produce heart defects in mice. In an elegant extension of this work, the region associated with heart defects has been further delineated. Mice carrying either a 5.43 Mb duplication or the corresponding deletion of a region extending from and including the *Tiam1* and *Kcnj6* genes, Dp(16Tiam1-Kcnj6)Yey/+ and Df(16Tiam1-Kcnj6)Yey/+, were produced by chromosomal engineering [83]. These experiments, including breeding the Dp(16)1Yey/+ mice with the Df(16Tiam1-Kcnj6)Yey/+ to restore disomy of the genes in this region, demonstrate that trisomy of the Tiam1-Kcnj6 region is necessary and sufficient to produce heart defects in mice. This approach is logically similar to the approach of breeding trisomic mice with knockouts of individual genes to assess the role of these genes in various DS phenotypes, described below.

3.1.7. Tc1 (Human Transchromosomal). A caveat of trisomic mouse models is the possibility that increased dosage ("trisomy") of HSA21 genes may produce different phenotypic effects. Some investigators have argued that a mouse in which HSA21 has been stably introduced into the mouse genome would be a better model of DS. In 2005, O'Doherty et al. [84] reported the production of a mouse carrying an HSA21 that was missing a small number of HSA21 genes. This HSA21 was reported to have about 91% of the full complement of HSA21 genes. The mouse has many features seen in individuals with DS. However, so far, all Tc1 mice are mosaics. That is, the chromosome is present in a variable number of cells in any tissue. More recently, it has been reported that the HSA21 in the Tc1 mouse only contains 81% of the full complement of HSA21 genes [85]. In addition, this chromosome apparently contains a duplication of the S100β and PRMT2 genes [30]. Tc1 mice lose the extra HSA21 chromosome on an inbred pure genetic background. Moreover, some of the phenotypes of these mice depend upon the genetic background of the animals. This is not a surprising result and provides another cautionary note regarding the use of mouse models.

3.2. Transgenic Mice. Transgenic mice contain additional, artificially introduced foreign genetic material, often a single gene, resulting in gain of function or overexpression of a certain protein(s). The use of transgenic mice provides an opportunity to study the biochemical and phenotypic implications of overexpression of individual trisomic genes *in vivo*. Molecular cloning of individual HSA21 encoded genes allows analysis of their expression and organization of their products and possible contributions to the DS phenotype. Features of transgenic mice that should be considered include gene copy number, levels of transcription and protein expression, tissue specificity and timing of expression, the site of integration of the transgene, and the genetic background of the mice. Figure 1 shows the location of these genes (indicated in bold) on HSA21 and the syntenic mouse chromosome regions.

3.3. Transgenic Mice Possibly Related to Intellectual Disability and Altered Brain Function in Individuals with DS. Several genes on HSA21 have been found to be important for neurodegenerative disorders, notably AD and amyotrophic lateral sclerosis (ALS, or Lou Gehrig's disease) or for synaptic function and neurological development and degeneration.

3.3.1. APP. APP (aka AAA, AD1, PN2, ABPP, APPI, CVAP, ABETA, PN-II, and CTFγ; App is the murine homolog) encodes the amyloid beta (A4) precursor protein, which is a cell surface receptor and transmembrane precursor protein. Multiple transcript variants encoding different isoforms have been found for this gene. It is overexpressed in some trisomic mouse models and in individuals with DS [86, 87]. APP is concentrated at the synapse in neurons and may play a role in synapse formation and plasticity [88–90]. Typically, APP undergoes extensive posttranslational processing including phosphorylation, glycosylation, and proteolysis. Normal APP proteolysis involves cleavage of the extracellular domain by an α-secretase followed by cleavage of the intermembrane domains by γ-secretase. The amyloidogenic pathway caused by abnormal cleavage of APP by β-secretase leads to aggregation of beta-amyloid peptide after cleavage by γ-secretase. The production of amyloid plaques is considered a hallmark neuropathological feature of AD. The protein is cleaved by secretases producing a number of peptides. Some of the peptides are secreted and bind to the acetyltransferase complex APBB1/TIP60

to promote transcriptional activation. Other peptides are components of the amyloid plaques found in the brains of patients with AD. Mutations in this gene have been implicated in autosomal dominant AD and cerebroarterial amyloidosis. Early in life, individuals with DS begin to develop progressive aggregation of beta-amyloid peptide and AD-like neuroanatomical features.

Initially, transgenic models overexpressing wild-type (WT) APP did not result in development of a neurodegenerative condition or AD-like pathologies such as amyloid plaques. Though there are several WT APP transgenic lines, only one appears to form plaques [91].

Using mice transgenic for WT human APP, Salehi et al. [92] demonstrate that increased APP expression results in a modest but significant decrease in nerve growth factor (NGF) transport.

Simón et al. [93] show that overexpression of WT APP in mice results in multiple pathological features, including cognitive deficits, severe histopathological abnormalities in cytoskeleton, and signs of synaptic dysfunction, as well as evidence of cell loss in the hippocampus and entorhinal cortex. These alterations are accompanied by an early increase in phosphorylated tau protein and elevated levels of APP derived carboxy-terminal fragments but, remarkably, almost undetectable levels of Aβ peptide. These results strongly suggest the presence of Aβ independent pathogenic pathways in AD.

The discovery of familial AD (FAD) mutations led to the overexpression of mutant APP in transgenic mice that does induce plaque pathology. These mice recreate many of the pathologies associated with AD, including early-onset AD as seen in DS. Overexpression of WT APP in DS is associated with early onset AD [94]. Recently, it was found that overexpression caused by APP gene duplication might lead to FAD [95–97].

Overexpression of APP in these models is often at levels far exceeding physiological levels, often up to 10-fold higher. It has been suggested that overexpression of APP, or any protein for that matter, at such high levels may be toxic. In humans, amyloidopathy often results in a progressive neurodegenerative condition; in mice this seldom is the case. Though amyloidopathy does cause cognitive decline, it is more reminiscent of natural aging or a predementia stage rather than a complete neurodegenerative disease (reviewed in [98]). In fact, the level of plaque load does not correlate well with severity of cognitive decline in people with AD [99].

The use of transgenic APP mouse models as models of AD as well as models of aging is further discussed [98, 100, 101]. For a general list of transgenic APP, as well as other models for neurodegenerative disease, the interested reader is referred to the Alzheimer's forum: http://www.alzforum.org/res/com/tra/.

3.3.2. RCAN1. The regulator of calcineurin 1 gene (RCAN1, aka CSP1, DSC1, RCN1, DSCR1, MCIP1, ADAPT78; Rcan1 is the murine homolog) encodes the calcipressin-1 protein, which interacts with calcineurin A, and inhibits calcineurin-dependent signaling pathways (such as activation of nuclear factor of activated T-Cells (NFAT) transcription factors) [102]. The gene is overexpressed in brain of DS fetuses. RCAN1 is up regulated by calcineurin signaling, suggesting regulation via a negative feedback loop. Calcineurin is an ubiquitously expressed Ca^{2+}-dependent phosphatase abundant in both the developing and adult brain, heart, skeletal muscle, and endocrine tissue [102–104]. It is responsible for many Ca^{2+}-dependent neuronal functions including neurotransmitter release, neurite outgrowth, cytoskeletal stabilization, and apoptosis (reviewed in [105]).

In trisomic mice, such as the Ts65Dn model, and in DS fetal tissue, RCAN1 is increased by up to 1.8 fold possibly affecting CNS development [40, 104]. In individuals with sporadic AD, RCAN1 is overexpressed in the cerebral cortex and hippocampus, and chronic overexpression may lead to neurofibrillary tangles associated with AD pathology [104]. Ca^{2+} induces the expression of RCAN1 in a calcineurin-dependent manner creating a negative feedback mechanism causing sustained calcineurin repression [106]. Therefore, the regulation of calcineurin by RCAN1 is of significant importance in the pathology of DS and AD.

Transgenic mice generated using a human RCAN1 cDNA splice variant 1 under the control of the endogenous promoter show a 4-fold increase in expression. Chromaffin cells taken from the transgenic mice show disruption in exocytosis and vesicle trafficking mechanisms in a non-calcineurin-dependent manner [107]. The authors make apparent that these results are from a transgenic model with a 4-fold increase in expression, which is much higher than the 1.5–2 fold increase typically found in individuals with DS.

A similar RCAN1 transgenic mouse was generated under the control of the platelet-derived growth factor beta (PDGFβ) promoter to drive expression in the brain [108]. These mice show a 1.3–1.5 fold overexpression in the hippocampus and cerebral cortex along with poor performance in the Morris water maze, indicating a disruption in visuospatial learning. However, no differences in performance of memory tasks were observed, suggesting once a task was learned, retention was not impaired [108]. The authors conclude that RCAN1 overexpression may contribute to a disruption in the calcineurin-dependent phosphorylation/dephosphorylation balance in the hippocampus and may inhibit learning, but not memory.

3.3.3. SYNJ1. Phosphatidylinositol-4, 5-bisphosphate is an important intracellular signaling phospholipid and plays essential roles in signal transduction, membrane trafficking, and cytoskeletal dynamics [109–111]. Because it plays a significant role in several cellular signaling events, balance at the cellular membrane is crucial. Phosphate kinase type-1γ (PIPK1) and synaptojanin 1 (SYNJ1) are critical to maintaining this balance at neuronal synapses [112, 113]. Synaptojanin 1 may act by dephosphorylating PtdIns(4,5)P_2 and may help stabilize PIPK1 [111, 113].

SYNJ1 is found on HSA21 and is trisomic in individuals with DS [87, 114]. Considering the vital role SYNJ1 plays in cellular dynamics through PtdIns(4,5)P_2 regulation, dysfunction of PtdIns(4,5)P_2 metabolism through SYNJ1

overexpression may result in neurophysiological changes seen in DS and may contribute to early onset AD pathology [115, 116]. Individuals with DS develop the pathology of AD by their 3rd decade, possibly due to the overexpression of APP and an increase in beta amyloid plaques [117]. Additionally, the overexpression of SYNJ1 due to trisomy of HSA21 may render neurons more sensitive to the insults of beta amyloid [116].

Transgenic mice were generated using BAC constructs for both human and mouse SYNJ1 genes [115]. The human and mouse SYNJ1 transgenic mice presented a 2.5-fold increase in transcript levels and a 59% and 38% increase in protein levels. Overexpression of SYNJ1 resulted in altered PtdIns(4,5)P_2 metabolism in the brains of these mice. These authors suggest that given the pleiotropic nature of PtdIns(4,5)P_2, irregularities in the metabolism of PtdIns(4,5)P_2 could have significant effects on many different cellular functions. In addition to the altered PtdIns(4,5)P_2 metabolism, these mice exhibit poor performance in the Morris water maze suggesting deficits in cognition and learning [115].

3.4. Transgenic Mice Possibly Related to the Altered Cancer Spectrum in People with DS. Individuals with DS have an altered spectrum of cancers. Specifically, there is a significantly increased risk of childhood leukemia and a significantly decreased risk of some solid tumors including many for which incidence is age related [118]. Some trisomic mice have similar features. Therefore, considerable work has been done with transgenic and KO mice with altered levels of genes encoded on HSA21 thought to be relevant to cancer.

3.4.1. ETS2. The v-ets erythroblastosis virus E26 oncogene homolog 2 (avian) (ETS2, aka ETS2IT1; Ets2 is the murine homolog) encodes protein C-ets-2, a transcription factor, and is a prototype of the ETS family of transcription factors. The gene for ETS2 is found on HSA21. The ETS family of transcription factors activate or repress genes responsible for cellular proliferation, differentiation, stem cell development, cellular transformation and tumorigenesis, cell senescence, and apoptosis [119]. The conserved ETS domain within these proteins is a winged helix-turn-helix DNA-binding domain that binds the core consensus DNA sequence GGAA/T of target genes [120]. Ets2 is essential for trophoblast development and is involved in establishing the AP axis and paraxial mesoderm during development.

Overexpression of Ets2 has been shown to increase apoptosis and is linked to DS pathophysiology [121–123]. Ets2 transcription factors are found in neurons and seem to be critical for neuromuscular junction formation in mice [124]. Mouse models with less than a 2-fold overexpression of Ets2 show neurocranial, viscerocranial, and cervical skeletal abnormalities reminiscent of trisomy 16 mouse models and individuals with DS [125]. This model expresses the Ets2 cDNA transgene under the control of a metallothionein promoter causing ubiquitous overexpression. These phenotypes are reminiscent of physiological conditions of individuals with DS and trisomic mice [125].

Using ETS2 transgenic mice, Wolvetang et al. [126] show that the Ets2 transcription factors activate the APP gene via specific Ets binding sites, acting cooperatively with the AP1 transcription factor. Furthermore, brains and primary neuronal cultures from ETS2 transgenic mice and from fibroblasts overexpressing ETS2 display abnormalities reminiscent of DS such as elevated APP protein and beta-amyloid production [126]. This may exacerbate the effects adverse effects caused by APP overexpression in individuals with DS.

3.4.2. RUNX1. The runt-related transcription factor 1 gene (RUNX1, aka AML1, CBFA2, EVI-1, AMLCR1, PEBP2aB, and AML1-EVI-1; Runx1 is the murine homolog) encodes runt-related transcription factor 1. RUNX1 is a hematopoietic transcription factor associated with normal hematopoiesis and megakaryopoeisis development [127, 128]. RUNX1 protein forms a heterodimeric transcription complex with core-binding factor β (CBFβ). This complex is the most common target observed in leukemia-associated translocations, suggesting that it has an important role in regulation of normal hematopoiesis. Children with DS are more likely to develop leukemia, and 10% of children with DS are born with transient megakaryoblastic leukemia (TML), which often develops into acute megakaryocytic leukemia (AMKL) [129]. RUNX1 is responsible for the terminal differentiation of megakaryocytic progenitors. Mutations, and translocations of RUNX1 are associated with acute myeloid leukemias (AMLs) [128]. However, trisomy of RUNX1 does not seem to be directly involved in TML or the progression of AMKL in DS [130–132].

Transgenic mice expressing mouse Runx1 under the control of the GATA1 hematopoietic regulatory domain (HRD) were generated to determine the role of Runx1 in the development myeloid leukemia in mice [133]. These mice show roughly a five-fold overexpression of Runx1 transcript and protein in whole bone marrow. It was determined that a 5-fold increase in Runx1 did not initiate an increase in leukemia. However, this group proceeded to cross the transgenic Runx1 mouse with the BXH2 mouse model of myeloid leukemia effectively adding an additional copy of Runx1. These mice show a decrease in the time period of myeloid leukemia onset [133]. The overexpression of Runx1 in the Runx1-BHX2 cross is reminiscent of childhood DS, AMKL, and similar to children with DS, this condition was preceded by TML. However, these mice show a 5-fold increase in Runx1 expression levels initiating TML and AMKL, which to date has not been reported in children with DS.

Interestingly, RUNX1 physically interacts with GATA1 [134]. GATA1 has been shown to be dysfunctional in children with DS and in the development of AMKL [135–138]. It has been suggested that an overdose of RUNX1 may render GATA1 dysfunctional, and this may lead to the development of AMKL in children with DS [139].

3.5. Mice with Genes Inactivated by Targeted Mutagenesis. Gene deletion is a powerful method for investigating gene

function, and for determining whether or not a gene is essential for viability. It is also useful for evaluating genes via manipulation of gene dosage in the context of two separate hypotheses; the "gene dosage effect," in which abnormal expression of individual genes is responsible for specific DS features, and "developmental instability," in which homeostasis is disrupted by chromosomal imbalance and aberrant expression of many genes, resulting in developmental abnormalities.

3.6. Knockout Mice Possibly Related to Intellectual Disability and Altered Brain Function in Individuals with DS

3.6.1. APP. An App null mutant was generated via gene targeting using a vector designed to replace the App promoter, exon 1, and part of the first intron with a neomycin phosphotransferase gene (PGKneo) cassette [140]. Neither App mRNA nor protein was detectable in App null animals, however, these mice are viable and do not display any overt abnormalities. Neuroanatomical analysis of brain tissue did not show any significant differences versus WT. However, Heber et al. [141] demonstrated that the App functions are indeed essential. App is one member of a gene family including amyloid beta (A4) precursor-like protein 1 and 2 genes (Aplp1 and Aplp2). They demonstrate that mice null for Aplp2 have no apparent abnormalities, but mice null for both App and Aplp2 exhibit perinatal lethality, indicating redundancy. They obtained similar results with mice null for both Aplp1 and Aplp2, which suggests a critical role for Aplp2. Mice null for both App and Aplp1 are viable. Surprisingly, mice null for both App and Aplp2 show no obvious histopathological abnormalities in brain, and cortical neurons showed normal survival in basal culture.

Salehi et al. [92] demonstrated that, in Ts65Dn, there is a marked decrease in nerve growth factor (NGF) transport in hippocampus, resulting in down regulation of nerve growth factor receptor (NGFR, or p75NTR) gene expression and deterioration of basal forebrain cholinergic neurons (BCFN). In Ts1Cje mice, there is a very mild decrease, when compared to euploid mice. The authors hypothesize that the marked decrease is due to trisomy of the App gene, which is trisomic in Ts65Dn, but not in Ts1Cje. Crossing Ts65Dn with a null allele for App (i.e., bringing the App gene dosage from trisomy to disomy) partially rescues the NGF decrease. Consistent with this observation, mice transgenic for a human APP allele, which expresses the gene at levels comparable to levels in DS, show a (relatively mild) reduction in NGF transport.

These observations suggest that trisomy of App is largely, but not exclusively, responsible for the decrease in NGF transport and the resulting reduction in BCFN seen in Ts65Dn. Mice transgenic for both APP and presenilin 1 (PSEN1, or PS1) show further reduction in NGF transport and in BCFN number, indicating an additive effect, further supporting this hypothesis. Early endosomal alterations are the earliest known pathology in sporadic AD and DS [142]. These alterations appear before birth in DS, and in AD, prior to the deposition of β-amyloid and as soluble Aβ levels first rise. The alterations have been observed in the hippocampus, neocortex, and basal forebrain.

The endosomal alterations are seen in Ts65Dn mice, but are not seen in Ts1Cje mice (which are disomic for App), or in Ts65Dn disomic for App (Ts65Dn, App+/+/−), which indicates that increased App expression is required for the alterations. However, the alterations are not present in mice transgenic for either the human APP London (APP670/671 plus APP717) or Swedish (APP670/671) mutations. Both mutations result in high expression of APP (two fold for the London transgenic, and seven fold for the Swedish transgenic). These results indicate, collectively, that App overexpression is necessary, but not sufficient for producing the alterations; overexpression of one or more additional MMU16 genes trisomic in Ts65Dn is required. The endosomal alterations may be at least partially due to reduction in NGF transport [143].

3.6.2. RCAN1. An Rcan1 null allele was generated by gene targeting using a vector designed to replace exons 5 and 6 with β-galactosidase [144]. Null (−/−) Rcan1 mice are viable and fertile, and exhibit no overt abnormalities. Northern blot analysis demonstrates that the null allele does not produce detectable transcript.

Calcineurin has been shown to be necessary and sufficient for cardiac hypertrophy, in response to various physiological and pathological stimuli. KO mice lacking the calcineurin A β catalytic subunit exhibit diminished response to hypertrophic stimuli. Since calcipressin-1 inhibits calcineurin-dependent signaling, increased expression of calcipressin-1 would be expected to reduce the hypertrophy response, and decreased expression should increase hypertrophy. Consistent with this expectation, Rcan1 null mice carrying a muscle-specific transgene expressing activated calcineurin showed an exacerbated hypertrophic response, and severe fibrosis. Unexpectedly, cardiac hypertrophy was reduced in null mice in which the hypertrophic stimulus was due to aortic banding, or chronic adrenergic stimulation. This suggests that calipressin-1 may have a dual role in cardiac hypertrophy, dependent on differences in hypertrophic stimulation.

Rcan1 is expressed in mouse in developing brain and craniofacial structures. It is trisomic in several DS mouse models, including Ts65Dn, Ts1Cje, and Ts16. Ts16 embryos exhibit high incidence of cardiac valvuloseptal malformations and abnormal development of the brain, skull, and sensory organs. However, mice trisomic for a Mmu16 region syntenic to an HSA21 region (Ts1Rhr [75]), but not including the Rcan1 locus (among other loci), do not develop cranial dysmorphologies, suggesting the possibility that overexpression of Rcan1 may be partially or fully responsible for these phenotypes.

Lange et al. [145] evaluated expression of Rcan1 in the Ts16 mouse model and show that expression of Rcan1 isoforms is increased in developing heart and brain, versus diploid littermates, while NFAT transcriptional activity is decreased. To evaluate the role of Rcan1 in Ts16 trisomy, they employed a breeding strategy using the Rcan1 null mice

[144] to restore the Rcan1 locus to disomy, in the Ts16 background. Examination of these mice demonstrates that restoring Rcan1 to disomy in Ts16 mice does not rescue cardiac and craniofacial abnormalities.

It has long been known that the incidence of many cancer types (typically solid tumors) is reduced in individuals with DS, and this protection is thought to be due to increased expression of one or more of the chromosome 21 genes that are trisomic in DS. RCAN1 suppresses vascular endothelial growth factor- (VEGF-) mediated angiogenic signaling via the calcineurin pathway. Baek et al. [40] demonstrated that RCAN1 is expressed about 1.8 fold higher in DS fetal tissues, and Rcan1 is expressed about 1.7 fold higher in Ts65Dn mice. They tested two tumor models, Lewis lung carcinoma and B16F10 melanoma, in Ts65Dn mice, and observed considerable tumor growth suppression relative to WT, accompanied by a decrease in microvessel density. They obtained similar results using an Rcan1 transgenic mouse, and also noted a significant decrease in $CD31^{+}CD45^{-}$ cells (CD31 is an endothelial marker, CD45 is a hematopoietic marker) versus WT. Inoculation of transgenic and WT mice with reduced numbers of Lewis lung carcinoma cells to generate slowly growing tumors demonstrated that increased Rcan1 expression inhibits the initial expansion as well as extended growth of transplanted tumors, indicating inhibition of both neoangiogenesis and co-option of existing blood vessels.

Matings were performed to produce Ts65Dn/$Rcan1^{+/+/-}$ mice, which exhibit significantly abrogated tumor protection along with increased microvessel density, demonstrating that Rcan1 overexpression plays an important role in these processes [40]. Since increased Rcan1 dosage attenuates VEGF-calcineurin-NFAT signaling, the authors examined the role of Dyrk1A, which is also trisomic in DS and Ts65Dn, and regulates NFAT signaling. They demonstrate that overexpression of both Dyrk1A and Rcan1 in endothelial cells results in greater inhibition of VEGF-mediated endothelial proliferation than in cells overexpressing Rcan1 alone, suggesting that Dyrk1A may be responsible for the increased tumor suppression observed in Ts65Dn/$Rcan1^{+/+/-}$ versus WT.

3.6.3. Synj1. A Synj1 null allele was generated by gene targeting using a vector designed to replace 103 base pairs (bp) from the 3′ portion of the first coding exon, and 1571 bp of the adjacent intron with a neomycin resistance cassette [109]. Mice heterozygous for the null allele are phenotypically normal and fertile. Crosses between heterozygotes produce pups with the expected genotypes, at the expected Mendelian ratio. However, within a few hours, the homozygous null pups become distinguishable from their littermates by the severe reduction in the amount of milk in their stomachs. About 85% of the homozygous null mice die within 24 hours, and the remaining 15% die within 15 days. The latter group exhibit reduced growth, with a 3-fold difference versus littermates at 10 days, and they develop severe weakness, ataxia, and generalized convulsions that can be evoked by the tail flick test. These results clearly indicate that the gene is essential for postnatal development. The absence of Synj1 expression did not alter the expression of a large variety of nerve terminal proteins, including synaptojanin 1 interactors, proteins thought to play a role in synaptic vesicle endocytosis, intrinsic membrane proteins of synaptic vesicles, plasma membrane t-SNAREs, additional proteins thought to play a role in the synaptic vesicle cycle, and enzymes involved in phosphoinositide (PI) metabolism.

The authors demonstrate a 1.6-fold increase of $PtdIns(4,5)P_2$ in cultured cortical neurons from null mice versus WT, but no major differences in PtdIns(4)P (they were unable to detect other PI species), and that the increase in $PtdIns(4,5)P_2$ is due to a reduction in dephosphorylation of $PtdIns(4,5)P_2$ to PtdIns(4)P. Electron microscopy of cultured cortical neurons showed an increased number of clathrin-coated vesicles localized around the synaptic vesicle cluster, and that the great majority are isolated vesicles (separated from the plasma membrane). The PI binding properties of clathrin coat proteins suggest that the increased number of clathrin-coated vesicles is due, at least in part, to increased $PtdIns(4,5)P_2$. Similar results were obtained in cell-free assays in which protein-free liposomes from crude brain lipid extracts were incubated in brain cytosol plus ATP and GTP. Cytosol from null animals produced a 4-fold higher number of coated vesicles than WT cytosol. Biochemical analysis showed a larger pool of clathrin and AP-2 bound to liposomes incubated with the null cytosol. This difference was counteracted by addition of purified synaptojanin 1 to the null cytosol.

Electrophysiological analyses of hippocampal slices from 10-day-old animals suggest that basal properties of synaptic transmission are unchanged in null mice, but that regeneration of a releasable pool for synaptic vesicle release is diminished in hippocampal synapses from null animals resulting in a depression of synaptic response. The authors suggest that the absence of synaptojanin 1 may affect actin dynamics as well (PI are important regulators of the actin cytoskeleton), resulting in trapping of clathrin-coated vesicles within an actin matrix.

The expression of Synj1 in Ts65Dn brain is about 40% greater than in controls as measured by quantitative western blot [115]. This overexpression in Ts65Dn results in a 33% increase in the production of phosphatidylinositol monophosphate (PtdInsP) relative to controls in a brain cytosol assay, using NBD-$PtdIns(4,5)P_2$, a fluorescently labeled water-soluble substrate. Reducing the copy number of Synj1 to disomy in Ts65Dn results in reduction of PtdInsP production to control levels. Similarly, HPLC analysis with suppressed conductivity detection demonstrates a ~16% decrease in the mass of $PtdIns(4,5)P_2$ in Ts65Dn brain relative to controls. The decrease was fully corrected in brain from Ts65Dn mice disomic for Synj1. Finally, in metabolic labeling studies of phospholipids in cortical synaptosomes, they demonstrate a ~30% decrease in the $PtdInsP_2$/PtdA ratio in Ts65Dn versus controls. The authors suggest that increased expression of Synj1 may play a role in the learning deficits observed in Ts65Dn mice. As stated above (Section 3.3.3), the authors demonstrate a learning deficit, as evaluated by the Morris Water Maze test, in mice transgenic for either murine Synj1 or human SYNJ1. Unfortunately,

they did not evaluate Ts65Dn mice disomic for Synj1, although they do state that performing this experiment is essential [115].

3.7. Knockout Mice Possibly Related to the Altered Cancer Spectrum in People with DS

3.7.1. ERG. The v-ets erythroblastosis virus E26 oncogene homolog (avian) gene (ERG, aka p55, and erg-3; Erg is the murine homolog) encodes transcriptional regulator ERG, a member of the erythroblast transformation-specific (ETS) family of transcription factors.

ERG is involved in chromosomal rearrangements in myeloid leukemia, in 5 to 10% of cases of Ewing's sarcoma, resulting in fusion of Erg and a member of the Tet subfamily of RNA-binding proteins. ERG is deleted in a subset of acute lymphoblastic leukemias, which may facilitate transformation, and is suggestive of a role for ERG in DS childhood leukemia. Chromosomal rearrangements result in control of ERG expression by the androgen-responsive 5′ elements of TMPRSS2 in more than half of all prostate cancers. Thus, there is strong evidence that ERG has an important role in hematopoiesis, and that it is a potent oncogene. So far, no ERG transgenic mice have been reported.

A germline mutation of Erg, designated Erg^{Mld2}, was obtained via a genetic screen for regulators of hematopoietic stem cell function [146]. Direct sequencing revealed that the mutation is a thymidine to cytosine transition in exon 12, causing a substitution of proline for serine at residue 329 in the first α-helix of the DNA-binding Ets domain. Pulse chase experiments in a human embryonic kidney cell line indicated the mutant protein has a half-life similar to that of WT Erg, suggesting that it is stable *in vivo*. Electrophoretic mobility-shift assays using radiolabeled DNA and titration with cold competitor DNA show that the mutant retains DNA binding ability and binds the E74 enhancer element (a known Erg-binding site) with an affinity similar to the WT protein. However, reporter assays demonstrate that the mutant's ability to transactivate transcription is negligible, and that it cannot promote megakaryocyte differentiation when expressed in human erythroleukemic cell line K562.

No $Erg^{Mld2/Mld2}$ mice from matings of mice heterozygous for the Erg^{Mld2} allele were identified at weaning, indicating the homozygous mice were probably dying during embryogenesis. Analysis of embryos from timed matings shows that homozygous mice are viable at day E10.5, some were dead at E11.5, and none were alive at E13.5. Homozygous embryos at day E10.5 exhibit developmental delay, and culture of yolk sacs from these embryos yielded almost no hematopoietic progenitor-derived colonies of any lineage, demonstrating failure of definitive hematopoiesis in the $Erg^{Mld2/Mld2}$ mice.

Mice heterozygous for the Erg^{Mld2} allele have lower blood platelet numbers than WT, but are not anemic. Histopathology of tissues from adult thymus, spleen, bone marrow, pancreas, lymph nodes, liver, kidney, bladder, small bowel, skin, skeletal muscle, salivary gland, or femur showed no gross abnormalities. Culture of single-cell suspensions of bone marrow and spleen yielded fewer colonies than WT, and the frequency of progenitor cells of all lineage types was about 50% that of control littermates. Colony-forming assays demonstrate that mice heterozygous for the Erg^{Mld2} allele have fewer committed hematopoietic progenitors and multipotent cells, and a smaller population of lineage-negative Sca-1^+c-kit^+ (LSK) cells (representing long-term repopulating hematopoietic stem cells and early hematopoietic progenitors).

Ts65Dn mice at 12 months of age exhibit progressive thrombocytosis, megakaryocytosis, and megakaryocytic dysplasia within bone marrow, extra medullary hematopoiesis in spleen with disrupted splenic architecture, and so forth [147]. Breeding the Erg^{MLD2} mutation into the Ts65Dn background to produce mice disomic for ERG results in amelioration of histopathologic myeloproliferative features to WT levels. Interestingly, Ts1Cje mice, although trisomic for Erg, do not develop myeloproliferative disorder, suggesting that Erg is necessary, but not sufficient, for the Ts65Dn myeloproliferative features.

3.7.2. ETS2. An Ets2 allele (ets2db1) was produced by gene targeting using a vector designed to replace all or part of three exons of the gene coding for the Ets2 DNA binding domain with pMC1NeoA [148], resulting in deletion of a critical portion of the gene (a large fusion transcript is observed), and production of a truncated protein that binds to an Ets2 antibody [149]. Mice homozygous for the allele are not obtained from heterozygous matings due to a defect in formation of extraembryonic tissues. They can be obtained via complementation using tetraploid embryos (which produce functional extraembryonic tissues) [150].

At birth, ets2db1 homozygous mice exhibit curly whiskers. After ~2 weeks of age, in addition to curly whiskers, the mice exhibit wavy hair, and a slightly rounded forehead. Whole mount analysis of skin shows misalignment of hair follicles, resulting in ingrown curly hairs that fail to penetrate the epidermis. Mice deficient for TGFα and mice with a point mutation in the EGF receptor have a similar whisker-hair-hair follicle phenotype. The mice are fertile, and lymphoid and myeloid cell development is not significantly different from WT.

An Ets2 hypomorphic allele (Ets2A72) was produced by knock in gene targeting, in which the threonine target of Erk phosphorylation is replaced by alanine [151]. Ets2A72 homozygous mice exhibit normal fertility and longevity. They do not develop the hair and hair follicle abnormalities found in rescued Ets2db1 homozygous mice. Histological analysis of 50 organs did not reveal any unusual abnormalities. Mammary gland development in females is normal.

Since Ets2db1 homozygous mice die due to placental insufficiency, Wei et al. [152] employed the Ets2A72 allele to produce an Ets1/Ets2 double "null" to investigate the role of both genes in the ras/Raf/Mek/Erk pathway and prevent rescue of the individual null phenotypes. Mutations in both genes result in abnormal angiogenesis in development, and full lethality by about day E14.5 (Ets2 nulls (db1 and fl) acting essentially the same as mutated Ets2 (A72)). Both genes promote epithelial cell survival in angiogenesis. Both

genes are proto-oncogenes and may act in endothelial cells to affect tumor angiogenesis.

Misregulation of ETS2 is associated with cancer, and some studies suggest that increased dosage of ETS2 in DS contributes to a reduced risk of cancer [126]. Ts65Dn mice are trisomic for Ets2. In an elegant series of experiments, Sussan et al. [153] demonstrated that trisomy of Ets2 in Ts65Dn and Ts1Rhr mice suppresses the occurrence of intestinal tumors when these mice are bred with the Apc^{Min} mice that have a highly increased incidence of intestinal tumors. These studies are consistent with the proposed role of ETS2 in reducing tumor incidence in DS. As mentioned above, Ts65Dn mice develop cranial skeleton and thymus anomalies. Similar anomalies were seen in transgenic mice that constitutively overexpress a processed Ets2 transcript under metallothionein promoters. To evaluate the role of native Ets2 in the craniofacial and thymus phenotypes of DS, Hill et al. [36] used these mice to show that the reduction in Ets2 expression in these mice does not rescue thymus abnormalities, and mostly does not rescue cranial skeleton abnormalities, except for mesoderm-derived elements (the superoinfero height of the occipital bone is reduced by 16% in Ts65Dn, Ets2+/− versus euploid but is reduced by 4% in Ts65Dn versus euploid). These experiments confirm a role for Ets2 in the suppression of tumors in DS, but Ets2 does not play a major role in skeletal or thymus abnormalities seen in Ts65Dn mice.

3.7.3. RUNX1. A Runx1 null allele was generated by gene targeting using a vector designed to replace the splice acceptor and first 20 bp of the exon encoding the central 52 amino acids of the Runt homology domain (RHD, required for DNA binding) with a hygromycin B cassette [127]. Homologous recombination also introduces stop codons in all three reading frames, ensuring production of a truncated protein.

Mice heterozygous for the null allele are apparently normal, exhibiting no difference in hematocrits, nucleated blood cell counts, white blood cell differentials, or distribution of peripheral blood lymphocyte subsets as analyzed by fluorescence-activated cell sorting analysis (FACS). However, homozygous nulls die during embryogenesis at about day E12.5. Morphological evaluation of E12.5 null embryos shows extensive hemorrhaging within the ventricle of the central nervous system and the vertebral canal, which appears to originate in the ganglia of the cranial nerves, extending into the third and lateral ventricles. Hemorrhaging was also observed in the pericardial space and peritoneal cavity in most null animals. At E11.5, about 87% of null embryos are viable, and are indistinguishable from heterozygous or WT embryos, except for a slight liver pallor. Microscopic examination of null embryo liver at day 11.5 indicates a complete absence of liver-derived hematopoiesis. No erythroid, myeloid, or megakaryocyte cells were identified, and only primitive nucleated erythrocytes were observed in vascular channels and hepatic sinusoids. Runx1 null embryonic stem cells can differentiate into primitive erythroid cells *in vitro*, but no hematopoietic colonies were obtained in cultures from yolk sac or liver from null embryos, demonstrating that Runx1 is essential for liver hematopoiesis.

Hematopoiesis was not well characterized in Ts65Dn mice, except for one report demonstrating decreased proliferation of $CD34^+$ cells *in vitro* [154]. Kirsammer et al. [38] investigated hematopoiesis in Ts65Dn mice and demonstrate that they develop highly penetrant progressive myeloproliferative disease characterized by thrombocytosis, mild anemia, extramedullary hematopoiesis, bone marrow fibrosis, and distorted stem and myeloid progenitor compartments, and they note that the phenotype resembles human chronic idiopathic myelofibrosis (the incidence of which increases with age [155]). To elucidate the role, if any, of increased expression of Runx1, they employed a breeding strategy involving the Runx1 null allele [127] to produce Ts65Dn mice disomic for Runx1. They conclude that increased dosage of Runx1 is not required for development of megakaryocytic hyperproliferation, extramedullary hematopoiesis, and reticulin fibrosis observed in Ts65Dn mice.

Carmichael et al. [156] investigated hematopoiesis in Ts1Cje mice which, as discussed above, are trisomic for a smaller region of Mmu16 than that in Ts65Dn mice. Ts1Cje exhibits a hematopoietic phenotype similar to that observed in Ts65Dn mice, except Ts1Cje mice do not show any sign of development of thrombosis or myeloproliferative disease. This suggests strongly that trisomy of one or more genes within the trisomic region unique to Ts65Dn is responsible for development of thrombosis and myeloproliferative disease, while the other hematopoietic abnormalities are largely caused by trisomy of genes in the trisomic region common to both Ts65Dn and Ts1Cje.

4. Conclusions

Recent progress in methods for producing genetically altered mice demonstrates that it is now possible, at least in theory, to produce mice trisomic for any gene found on HSA21 or any mouse chromosomal region syntenic to HSA21, and KO mice for any HSA21 syntenic gene(s). Indeed, a large international effort is underway to produce KO mice for all mouse genes and to assess their phenotypes. Moreover, and equally important, it is possible to completely characterize the genetic alterations in the various mouse models, including alterations in gene number, expression, and structure, which is essential for proper interpretation of the consequences of trisomy of particular genes or chromosomal regions. This capability presents an unprecedented opportunity for unraveling the mechanisms of DS pathogenesis and, on the basis of this information, devising rational therapies for alleviation of the deleterious consequences of Trisomy 21.

The analysis of various mouse models to date allows some preliminary conclusions. By far, the most well-characterized mouse model phenotypically is the Ts65Dn mouse. Considerable evidence suggests that Ts65Dn mice exhibit aspects of aging relevant to DS. Interestingly, even though Ts65Dn mice are trisomic for about 60 Mmu17 genes and are disomic for about 16 Mmu16 genes found on

HSA21, their phenotype is remarkably similar to that of the Dp(16)1Yey/+ mice that are trisomic for the entire Mmu16 region syntenic to HSA21, and that have no additional trisomic genes. Of course, differences may be revealed as the mice are more thoroughly characterized. This observation does not mean that HSA21 genes found on Mmu10 or Mmu17 are irrelevant for DS. For example, the Mmu10 or 17 regions may ameliorate some of the effects of trisomy of the Mmu16 region.

In general, analysis of transgenic and KO mice reveals phenotypes consistent with a given gene's known function and in some cases have helped in elucidating its function. Also, when KO mice have been bred with trisomic mice, reducing a gene's copy number from three to two, the observed effects have been consistent with the gene's function as determined by other studies.

Often, the point has been brought up that the genetic background of the various mouse models is critical, since response to trisomy may differ depending on this parameter. One should keep in mind, however, that people with DS are certainly not inbred, and an effect seen in a noninbred mouse strain or in multiple genetic backgrounds may be more relevant to the human situation than effects observed in inbred models. It is also important to assess phenotypes in more than one mouse model where possible.

5. Future Directions

The results from studies of genetically altered mice, coupled with the ability to produce essentially any mouse model, demonstrate that this approach will play a key role in understanding the DS phenotype, as well as phenotypes related to the biology of aging in people with DS. Studies so far make it clear that the genetic alterations in mouse models can be precisely defined with regard to gene content and alterations in gene expression. Results from studies in which dosage of specific genes in segmentally trisomic mice is reduced to disomy via breeding with relevant KO mice suggests that this is a particularly fruitful approach. As KO mice for more genes become available, the pace of these experiments should accelerate. This approach appears to be more successful in revealing a gene's role in DS than the creation of single-gene transgenic mice. However, the production of transgenic mice to assess the effects of trisomy of specific genes, especially genes associated with a particular disorder, or for which a particularly compelling hypothesis suggests they may have a significant effect, may be worthwhile. Also, there may be strong justification for increasing the dosage of syntenic Mmu10 and 17 genes to trisomy in mice trisomic for Mmu16 regions.

The observation that segmental trisomy for Mmu10 and Mmu17 regions syntenic to HSA21 have relatively minor phenotypic effects appears to limit the regions of HSA21 important in DS. However, this interpretation may not yet be warranted. First, it is possible that continuing characterization of the various segmental trisomy mice may reveal phenotypes relevant to DS that are caused or affected by genes located in these regions. Moreover, it may be that trisomy of these regions interacts with trisomy of the Mmu16 syntenic region, affecting the Mmu16 trisomic phenotype.

Ts65Dn and other segmental trisomy mouse models serve as treatment models for some aspects of DS, and agents showing a beneficial effect in these mice are either in human clinical trials or will be soon on the basis of their success in ameliorating the symptoms in these models. This brings up an interesting and critical question, namely, is the approach of using particular mouse models valid in preclinical studies, especially when the mechanism of action of a given agent is poorly understood? One could argue that if a particular agent improves a deleterious phenotype reminiscent of those seen in people with DS, this should be sufficient justification for proceeding with human trials. This perspective is problematic in some ways. For example, it is possible that genes on the HSA21 syntenic Mmu10 or 17 regions will influence the result of drug treatment. For example, individuals with DS show an increased sensitivity to cytosine arabinoside and this may help explain the high event-free survival rates seen in treatment of people with DS who have developed AML. This increased sensitivity is attributed, at least partially, to trisomy of the CBS gene, located on Mmu17 in mice. Similarly, individuals with DS show a significantly increased sensitivity to methotrexate, one of the most widely used anticancer drugs, and this may be due to trisomy for the Slc19a1 (reduced folate carrier) gene, which is on Mmu10 [15]. It would be interesting to determine whether making Ts65Dn mice trisomic for CBS or for RFC would increase their sensitivity to cytosine arabinoside or methotrexate, respectively. The appropriate transgenic mice already exist and have been partially characterized. In general, the more one knows about the mechanism of drug's action, the more effectively one may be able to test it in appropriate mouse models. Therefore, mechanistic studies of the effects of possible therapies using mouse models would be extremely worthwhile.

It is reasonable to expect that therapies to improve intellectual and other disabilities associated with DS and/or aging will soon become available. Mouse models of DS will have played a critical role in this development, and it is virtually certain that they will continue to do so. Thus, mouse models will have made a major contribution to the lives of individuals with DS and their families. Moreover, any feature seen in individuals with DS is also seen in the population without DS. Therefore, use of these mouse models will likely have beneficial effects far beyond the population with DS.

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Review Article

Neuroinflammation in the Aging Down Syndrome Brain; Lessons from Alzheimer's Disease

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Down syndrome (DS) is the most genetic cause of mental retardation and is caused by the triplication of chromosome 21. In addition to the disabilities caused early in life, DS is also noted as causing Alzheimer's-disease-like pathological changes in the brain, leading to 50–70% of DS patients showing dementia by 60–70 years of age. Inflammation is a complex process that has a key role to play in the pathogenesis of Alzheimer's disease. There is relatively little understood about inflammation in the DS brain and how the genetics of DS may alter this inflammatory response and change the course of disease in the DS brain. The goal of this review is to highlight our current understanding of inflammation in Alzheimer's disease and predict how inflammation may affect the pathology of the DS brain based on this information and the known genetic changes that occur due to triplication of chromosome 21.

1. Introduction

Down syndrome (DS) is the most common chromosomal anomaly among live-born infants and is the most frequent genetic cause of mental retardation [1, 2], with an incidence of one per 733 live births in the United States [3]. DS is caused by a triplication of chromosome 21 (a full list of genes located on chromosome 21 can be found in [4]). Due to the extensive number of genes triplicated, there is an extremely high incidence of congenital cardiac and gastrointestinal abnormalities [5]. DS is usually detected during pregnancy through first-trimester screening tests followed up by confirmation through amniocentesis, chorionic villus sampling, or percutaneous umbilical blood sampling [6].

Alzheimer's disease (AD) is the leading cause of dementia and is characterized clinically by a progressive loss of memory and cognition. An absolute diagnosis of AD can only occur after pathological analysis is performed on the brain tissue. There are two signature pathological lesions required for diagnosis; neuritic plaques composed of aggregated amyloid-β (Aβ) peptides, and neurofibrillary tangles composed of hyperphosphorylated, aggregated tau protein [7]. AD is usually considered a disease of aging, where currently 1 in 8 Americans over the age of 65 have AD yet half of those over 85 years have AD (data obtained from the Alzheimer's Association; www.alz.org).

In DS, Aβ deposits begin to accumulate in childhood and increase progressively with age [8]. There is an acceleration of this pathology between the ages of 35–45 years when other AD pathologies begin to occur, most importantly neurofibrillary tangles and inflammation [9]. Despite the certainty of developing AD-like pathologies in DS by mid-life, the onset of dementia is less certain. The consensus from a number of studies is that 50–70% of DS individuals will develop dementia by ages 60–70 years [10–13]. The reason individuals with DS develop Aβ deposits early in life is primarily due to the presence of some AD-related genes on chromosome 21, and hence these genes are triplicated in most cases of DS. Of the AD-associated genes triplicated in DS, the critical ones are amyloid precursor protein (APP) and β-amyloid cleavage enzyme 2 (BACE2). Aβ peptide is a cleavage product of APP. APP is a transmembrane protein and is differentially cleaved by enzymes called secretases of which there exist α-secretase, β-secretase (BACE), and γ-secretase. When β-secretase and γ-secretase cleave APP Aβ is

a product, when α-secretase cleaves, this occurs in the middle of the Aβ portion and other peptides are produced.

Inflammation is known to occur in the brains of both AD and DS patients in response to the presence of neuritic plaques and neurofibrillary tangles. This inflammation is primarily mediated by microglial cells, although other glial cells and even neurons participate in this inflammatory response. It is becoming increasingly clear in the AD field that inflammation can directly influence plaques and tangles in the same way that plaques and tangles can directly influence inflammation. The purpose of this review is to discuss the evolving understanding of neuroinflammation in AD and determine how this may relate to the pathophysiology of DS.

2. Neuroinflammation in Alzheimer's Disease

Neuroinflammation is a complex process with many phenotypically varied states. The primary inflammatory cell in the brain is the microglial cell, which was first identified as a unique cell subtype by Del Rio Hortega in the 1920s. The microglial cell has been described as an ameboid-like cell that can be labeled immunocytochemically using macrophage cell surface markers [14, 15]. Other cells in the brain can contribute to the inflammatory response as well as microglia, although this contribution is considered to be significantly less than that of the microglia. Astrocytes and neurons can participate in the neuroinflammatory process as well as oligodendrocytes and vascular pericytes [16].

The view of neuroinflammation in the brain, and in disorders of the brain, has evolved over time, and continues to evolve as our understanding of the capabilities of the system grows. While once considered "immunologically privileged," the brain is now known to exhibit an almost complete spectrum of inflammatory responses given the correct stimuli and environment. While once considered a cytotoxic loop [17], there are now examples of harnessing the inflammatory system of the brain to ameliorate AD pathologies and improve outcomes (see further discussion later in this section).

In AD, microglia expressing some classic activation markers such as MHC-II (associated with antigen presentation), CD68 (a lysosomal protein), and CD36 (a class B scavenger receptor) are highly localized to the area immediately surrounding an amyloid plaque or neurofibrillary tangle [18]. While this led some to hypothesize that this reaction was contributing to the toxicity of these pathologies, others suggested that the microglia may be performing a beneficial function in removing the abnormal protein deposits from the brain. As yet, there is no consensus, and it is likely that both phenomena are occurring to differing degrees. To better understand these processes, researchers turned to the assessment of cytokines to determine the function(s) of these microglial cells.

In AD, many cytokines have been found to be altered. Among those, the most common are IL-1β, IL-6, TNFα, and TGFβ. IL-1β was first shown by Griffin et al. in 1995 to be associated with the development of neuritic amyloid plaques from diffused deposits using human postmortem tissue [19]. Later, Griffin et al. expanded their findings to develop a "cytokine cycle" hypothesis that suggested the IL-1β production in response to amyloid deposits initiated a series of events including increased APP production and processing by neurons, recruitment of astrocytes, and activation of these astrocytes leading to signaling in the microglia inducing yet further IL-1β [20]. IL-1β induces S100β production in astrocytes [21], which is a cytokine that promotes neurite growth [22]. Most recently, serum IL-1β has been found to be elevated in cases of mild cognitive impairment (MCI) that has a higher risk for conversion to dementia, possibly indicating that serum IL-1β may be useful for identifying those MCI patients at risk for converting to AD [23]. Also, there are genome-wide association studies (GWASs) that have identified IL-1β polymorphisms associated with AD (reviewed in [24]). It will require further studies and analyses to determine whether these polymorphisms, are, in fact, associated with AD risk. However, in contrast to the negative data presented with respect to IL-1β, there is more recent data showing that IL-1β overexpression in the hippocampus of transgenic mice results in amelioration of amyloid pathology. IL-1β was increased specifically in a single hippocampus of an APP/PS1 transgenic mouse by genetic means and this hippocampus showed a 50% reduction in plaque load [25].

IL-6 is another cytokine that mediates immune responses and inflammatory reactions [26]. While microglia are the main source of IL-6 in the CNS, astrocytes, neurons, and endothelial cells are all capable of producing the cytokine [27–29]. In AD, brain tissue IL-6 has been shown to be elevated in pathologically relevant regions [30]. While much of the focus on IL-6 has been on its destructive effects such as induction of acute-phase proteins, increasing vascular permeability, activation of lymphocytes, and antibody synthesis (reviewed in [31]), there are some positive effects of IL-6 that may play a role in AD. This includes enhancing neuronal survival [32–34] and suppressing demyelination in a model of multiple sclerosis [35]. Moreover, in a mouse model of amyloid deposition, Chakrabarty et al. showed that overexpression of IL-6 enhanced microglial phagocytosis of amyloid deposits and, therefore, ameliorated amyloid burden [36].

TNFα is another cytokine that has been shown to have both beneficial and detrimental effects in the CNS. It acts as a highly potent proinflammatory and cytotoxic molecule in conditions of the CNS [37–40]. In contrast, TNFα has been shown to have trophic effects on hippocampal neurons [41] and provide protection from free-radical damage in primary neurons [42]. It is thought that the source of such dichotomous effects is the receptor subtype through which the TNFα is acting. There are two primary receptors for TNFα in the CNS; TNFα receptor 1 (TNFR1) and TNFα receptor 2 (TNFR2) [43]. TNFR1 mediates neuronal death via the TNF-receptor-associated death domain protein and caspase-8-activated apoptosis [44, 45]. TNFR2 is thought to mediate the beneficial, prosurvival action of TNFα through the nuclear factor-κB- (NFκB-) mediated antiapoptotic pathway [46]. This is likely an oversimplified view of the actions of TNFα through its receptors and there have been many

subtleties of these systems described in the literature. In AD, it has been shown that expression of TNFR1 is elevated in the brain while levels of TNFR2 are decreased [44]. In addition, clinical trials are ongoing for the treatment of AD with etanercept, a fusion protein combining TNFR2 and the Fc portion of IgG used to treat Crohn's disease and arthritis as well as other autoimmune disorders [47]. Etanercept acts as a decoy receptor for TNF, reducing the effects of TNF at the biologically active receptors. Preliminary studies showed that perispinal delivery of etanercept in a small number of AD patients improved cognition [48]. In addition, thalidomide is also currently in clinical trials for AD based on its anti-TNFα effects. In transgenic mice, thalidomide has been shown to improve learning and memory [49].

Finally, TGFβ is a growth factor that has been shown to play a prominent role in tissue development, homeostasis, and repair [50]. Unlike the cytokines discussed to this point, TGFβ is associated mostly with repair mechanisms and is not known for its damaging or cytotoxic actions in the CNS. Instead, it is mostly associated with the formation of a glial scar [51] and upregulation of extracellular matrix proteins [52–54]. In AD, TGFβ levels are increased in the brain [55] but decreased in serum [56]. In APP transgenic mice, overproduction of TGFβ by astrocytes results in lower parenchymal amyloid deposits but increased deposition of amyloid in the cerebrovasculature [57]. Most recently, Tesseur et al. have shown that deficiencies exist in TGFβ signaling in the human AD brain, and these deficiencies can lead to enhanced AD pathology and associated neurodegeneration [58].

There is a rapidly growing interest in better characterizing the inflammatory state in the brain, and especially in AD. A paper by Colton et al. in 2006 described "classical activation" and "alternative activation" of microglia in the brain [59]. Classical activation was used to describe the Th1 cytokines such as IFNγ, IL-1β, TNFα, and IL-6. Alternative activation was used to describe a state associated with anti-inflammatory, repair, and wound healing effects mediated by IL-10, TGFβ, IL-4, IL-13, arginase 1 (AG1), and tissue remodeling factors Found in Inflammatory Zone 1 (FIZZ1) and chitinase 3-like 3 (YM1). This paper showed that cultured microglial cells, transgenic mouse models of AD, and postmortem tissue from human AD brains all showed expression of both classical and alternative activation markers. Most interesting was that alternative activation markers were expressed to the same degree, sometimes more than the classical activation markers commonly associated with an inflammatory response.

We have now expanded on the concept of multiple activation states to include a full spectrum of macrophage responses. Shown in Figure 1 are the four distinct inflammatory states we are currently studying in the brain. These states are well characterized in the peripheral macrophage literature (reviewed in [60, 61]). The M1 response is stimulated by IFNγ and/or TNFα and is characterized by traditional inflammatory cytokines IL-1β, IL-6, and IL-12. Broadly, the M2 response represents the alternative activation state described by Colton et al. We can further categorize this state into M2a, M2b, and M2c. Each subtype of M2 response has distinct stimuli and responses. IL-4 and/or IL-13 initiate an M2a response that is characterized by tissue remodeling factors FIZZ and YM1 as well as AG1 and mannose receptor C1 (MRC1). Immune complexes stimulate an M2b response, which is a specific response that has components of both M1 and M2a states. Finally, IL-10 stimulates an M2c response, which is sometimes called an acquired deactivation state. The M2c response is characterized by a series of markers that actively antagonize M1 signaling pathways. By categorizing the inflammatory response into these distinct types where each stimuli and marker is established, we can better understand what role(s) each state plays in AD progression and therapy.

Drug development for the treatment of AD has recently been harnessing the inflammatory component of the disease for treatment. The most interesting approach is immunotherapy for AD. First demonstrated in 1999 [62], immunotherapy uses either an active vaccination approach or passive immunization to introduce anti-Aβ antibodies in patients (reviewed in [63]). These anti-Aβ antibodies then result in reductions in Aβ in the brain and ultimately, at least in transgenic mouse models, improvements in learning and memory [64, 65]. Injection of anti-Aβ antibodies directly into the brains of transgenic mouse models showed a dependence of amyloid removal on microglial activation [66, 67]. Later studies systemically administering anti-Aβ antibodies also showed a transient activation of microglia [68] and a reduced efficacy when the antibody was deglycosylated; a process that renders the IgG molecule incapable of interacting with effector cells such as microglia [69].

Another approach that targets the inflammatory response is the administration of nonsteroidal anti-inflammatory drugs (NSAIDs). NSAIDs showed great promise in retrospective epidemiological studies finding significant protection from AD with long-term NSAID use [70]. However, a prospective clinical trial performed by NIA/NIH, called the ADAPT trial, failed to show any significant benefit [71]. The NSAID story was furthermore clouded because some NSAIDs also possessed γ-secretase modifying properties that shifted APP cleavage to promote Aβ38 production, as opposed to Aβ40 or Aβ42 [72]. The NSAIDs found to have this activity were not included in the NIA/NIH trial. However, it was recently found that a subset of patients in the ADAPT trial did, in fact, benefit from NSAID use. Naproxen attenuated cognitive decline in a subgroup of AD patients termed "slow decliners," whereas cognitive decline was accelerated in those termed "fast decliners" [73]. It is unclear why this would be the case, however, it is possible that different inflammatory states may exist in these different AD cases; some benefit from NSAIDs while some do not. Future studies will examine whether this is, indeed, the case.

3. Neuroinflammation in Down's Syndrome

While many of the pathways of inflammation described for AD will be directly relevant to DS, there are some critical inflammatory genes on chromosome 21 that will be triplicated in DS and may, therefore, influence the inflammatory

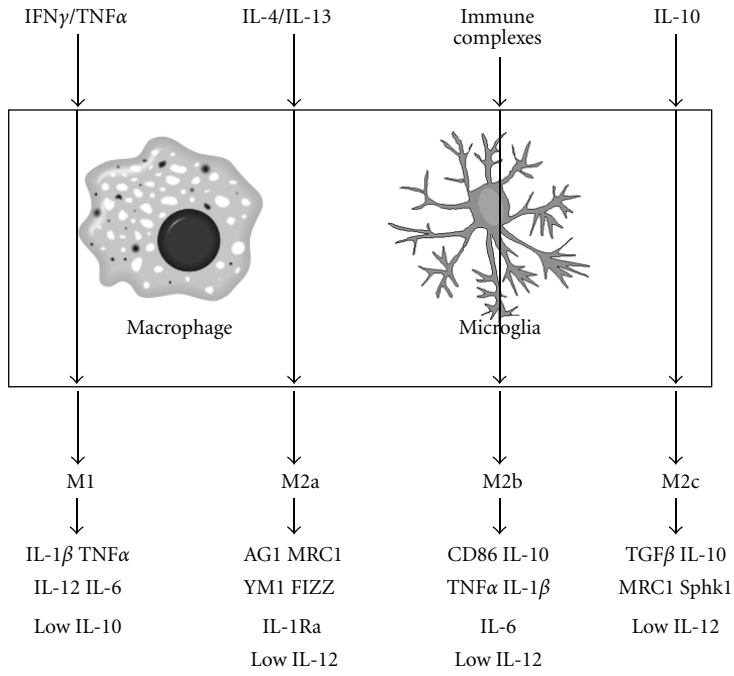

Figure 1: Schematic showing the four distinct states of inflammation possible in response to a stimuli in microglial/macrophage cells.

state of the DS brain. We will discuss those factors in this review and take our current knowledge of inflammatory states in other neurological disorders to predict how these may be playing a role in DS. Table 1 shows the inflammatory-associated genes that are found on chromosome 21 and are triplicated in most DS patients. We will discuss each of these factors and their impact on the inflammatory balance of the brain.

CXADR is a gene encoding for a protein called coxsackie virus and adenovirus receptor (herein abbreviated CXADR). CXADR has a dual function as a viral receptor and an adhesion molecule associated with tight junctions. It is highly expressed in brain as well as systemic secretory organs such as the pancreas, testis, and small intestine [74]. In the heart, CXADR is increased in models of myocardial inflammation and cardiac injury in the absence of viral infection suggesting that there is an innate role of this protein in the inflammatory response [75]. Recently, it was shown that CXADR can induce stress-activated mitogen-activated protein kinase (MAPK) pathways in the heart leading to increased production of IFNγ, IL-12, IL-1β, TNFα, and IL-6 [76]. One can predict then that increased expression of CXADR in Down's syndrome may contribute to an overactivated M1 inflammatory response, since all of these inflammatory cytokines induced by CXADR are associated with an M1 response. In addition, CXADR has a significant role in tight junction function where, in endothelial cells, it facilitates transendothelial migration of neutrophils [77]. If CXADR expression is altered on the endothelial cells of the cerebrovasculature in DS patients, then there may be altered infiltration of peripheral inflammatory cells into the brain influencing the inflammatory response.

Two members of the ADAMTS (a disintegrin and metalloproteinase with thrombospondin motif) family are located on chromosome 21 and, therefore, subject to triplication in DS, ADAMTS1, and ADAMTS5. ADAMTS1 contains a signal peptide in the N-terminal region indicating it is secreted [78]. It acts as a proteinase degrading extracellular matrix proteoglycans such as aggrecan and versican [79]. ADAMTS5 is also a proteinase and shares the same substrates as ADAMTS1 [80]. Both ADAMTS1 and ADAMTS5 can be induced by IL-1β, indicating a dependence on an inflammatory response [81, 82]. It has been shown in DS that ADAMTS1 is five-fold overexpressed at the protein level, while ADAMTS5 was not significantly increased by Western blot measurements [83]. Given the induction by inflammatory cytokine IL-1β, one could hypothesize that the triplication of these proteinases would lead to exacerbated degradation of extracellular matrix proteins in response to an inflammatory insult. In addition, Griffin et al. showed that DS brain has greater IL-1β immunoreactivity indicating that there is more IL-1β present in the DS brain to stimulate the ADAMTSs [84].

T-cell lymphoma invasion and metastasis 1 (TIAM1) is a guanine nucleotide exchange factor for Rac1 [85] and, therefore, contributes to the activation of Rac1, which is necessary for the activation of NADPH oxidase [86]. Most recently, Tiam1 was found to be a critical regulatory factor in cytokine-induced induction of NADPH oxidase, more specifically, induction by IL-1β [87]. While these data used pancreatic β-cells, one could predict that overexpression of Tiam1 in the DS brain could lead to increased oxidative stress in response to an inflammatory insult that involves IL-1β. Indeed, it has been shown that Tiam1 protein expression is

Table 1: A summary of the inflammation-related genes located on chromosome 21.

Gene	Protein	Function	Ref
CXADR	Coxsackie virus and adenovirus receptor	Activation of JNK and p38-MAPK pathways leading to production of M1 cytokines.	[76]
ADAMTS1	ADAM metalloproteinase with thrombospondin type 1 motif, 1	Secreted protease known to be induced by IL-1β	[81]
ADAMTS5	ADAM metalloproteinase with thrombospondin type 1 motif, 5	Secreted protease known to be induced by IL-1β and TGFβ.	[82]
TIAM1	T-cell lymphoma invasion and metastasis 1	Necessary for cytokine-mediated generation of oxidative species through NADPH oxidase.	[87]
SOD1	Superoxide dismuatose 1	Scavenges superoxide radicals producing H_2O_2 and O_2.	[109]
IFNAR2	Interferon (alpha, beta, and omega) receptor 2	Activates JAK/STAT-mediated pathway in response to IFNα/β.	[110]
IFNAR1	Interferon (alpha, beta, and omega) receptor 1	Activates JAK/STAT-mediated pathway in response to IFNα/β.	[110]
IFNGR2	Interferon gamma receptor 2	Activates JAK/STAT-mediated pathway in response to IFNγ.	[111]
RIPK4	Receptor-interacting serine-threonine kinase 4	Necessary for signaling through TNFR1	[96]
CBS	Cystathione-beta-synthase	Production of hydrogen sulfide (H2S); a regulator of inflammation	[112]
S100B	S100 calcium binding protein B	Constitutive expression by astrocytes, released in response to TNFα	[113]
PRMT2	Protein arginine methyltransferase 2	Blocks the actions of NFκB in the nucleus	[114]

increased in fetal DS brain compared to control fetal brain [88].

Superoxide dismutase 1 (SOD1) binds copper and zinc and is a potent endogenous antioxidant. The enzyme is a soluble cytoplasmic and mitochonidral interspace protein that converts superoxide radicals to molecular oxygen and hydrogen peroxide [89]. Mutations in the SOD1 gene are commonly associated with genetic susceptibility to anterolateral sclerosis (ALS) [90]. While the hypothesis for the role of these mutations centered on the potential loss of function, and, therefore, increased oxidative stress, there has been increasing evidence to discount this hypothesis including the lack of ALS symptoms or pathology in SOD1 knockout mice [91]. It is unclear what the consequence is of overexpression of nonmutant SOD1 as would occur in DS. In a model of retinitis pigmentosa, it was found that loss of SOD1 worsened the outcomes. However, when SOD1 was overexpressed in this model, the levels of oxidative damage were actually worse. The authors found that in the absence of a peroxide-detoxifying enzyme in the same cellular compartment, overexpression of SOD1 actually causes more oxidative stress [92]. It could be suggested that the same may be the case in DS if the triplication of SOD1 results in overexpression of the protein in the absence of an increased level of peroxide-detoxifying enzyme.

Interferon receptors IFNAR1, IFNAR2, and IFNGR2 are all located on chromosome 21 and are, therefore, all subject to triplication in most cases of DS. IFNAR1 and IFNAR2 both respond to IFNα, IFNβ, or IFNo and, upon ligand binding, activate the JAK/STAT signaling pathway leading to induction of proinflammatory gene expression such as IL-1β, TNFα, and IL-6. IFNGR2 uses the same signaling pathway but responds to IFNγ specifically. A mouse model for the study of DS, the trisomy 16 mouse, includes triplication of IFNGR2 and IFNAR2. These mice develop significant pathology *in utero* and rarely survive to birth. Studies in these mice have shown that anti-IFN IgG treatment of fetuses improves the mouse phenotype suggesting the triplication of the IFN receptors significantly contributes to the severe pathology present in these mice [93]. The same group later showed that introducing a partial knockout of the IFNAR2 and IFNGR2 can improve growth and viability of cultured neurons derived from the trisomy 16 mouse fetuses [94]. Since these genes are triplicated in DS, it is likely that there is a hyperresponsiveness to IFN in the DS patient that may lead to an increased inflammatory response, both in the brain and systemically.

Receptor-interacting serine-threonine kinase 4 (RIPK4) is a protein kinase involved in multiple cell signaling pathways. One of these pathways is the signaling pathway for the activation of NFkB [95]. In addition, RIPK4 is involved in the signaling cascade of the TNFα receptor TNFR1 [96]. It is important to note that the TNFR1 is most heavily implicated with the toxic effects of TNFα and it could be predicted that overexpression of RIPK4 may increase responsiveness of TNFR1 to TNFα exacerbating the effects of TNFR1. At this time, however, this is purely speculative.

Cytathione beta synthase (CBS) is a cytosolic enzyme that catalyzes the desulfhydration of cysteine-producing hydrogen sulfide (H_2S). H_2S is now recognized as an atypical cellular messenger that has many normal physiological functions [97]. CBS binds NO or CO in its heme pocket and

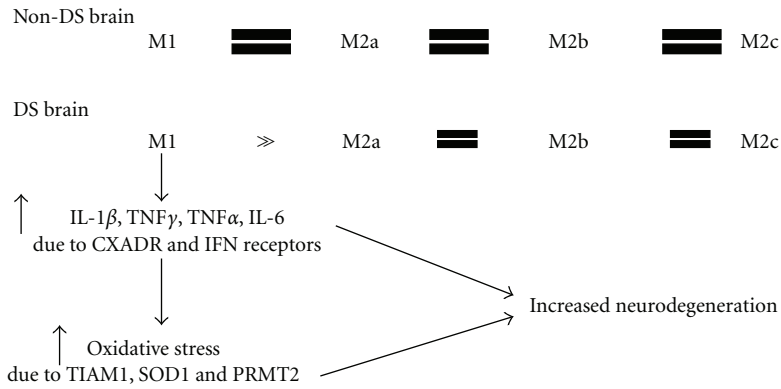

Figure 2: Schematic illustrating our hypothesis for the role of inflammation in Down syndrome.

this binding modulates the activity of the enzyme [98]. H_2S is a complicated signaling molecule with an apparent bimodal action on inflammation, where low levels appear to be anti-inflammatory, yet high levels may exacerbate inflammation in some instances. There are several extensive reviews on H_2S signaling that discuss this phenomenon in great detail (see [99, 100]). It remains unclear how the overexpression of CBS in DS influences the DS pathology and whether the amount of H_2S produced in DS patients is of the anti-inflammatory or proinflammatory concentrations.

S100β is a protein localized primarily to the brain where it is expressed by astrocytes. It is secreted by astrocytes in response to IL-1β and cyclic-AMP [101]. S100β is another inflammatory mediator with dichotomous actions. At low concentrations, it appears to enhance survival of neurons [102] and stimulate neurite outgrowth [22]. In contrast, high concentrations of S100β increases cell death [103] and causes apoptosis [104]. It has been shown in DS brains that S100 is greatly increased compared to control brain. The concentrations would place the levels of S100β in the toxic category, suggesting that the overexpression of S100β in DS brain plays a negative role in the aging pathology [84].

Protein arginine methytranferase 2 (PRMT2) is an en-zyme that catalyzes the methylation of arginine. It has been shown that arginine methylation is a means of regulation of the JAK-STAT signaling pathway, which is key for many inflammatory processes including IFNγ, IFNα, and IL-6 [105]. In addition, natural degradation of proteins containing methylated arginine results in the production of asymmetric dimethylarginine (ADMA) [106]. ADMA is an endogenous inhibitor of nitric oxide synthase (NOS), a key player in normal cell signaling and inflammation [107]. It is unclear whether the triplication of PRMT2 results in significant changes in ADMA concentrations in the brain, however, DS patients with pulmonary hypertension do show increased ADMA concentrations compared to non-DS patients with pulmonary hypertension [108]. If this were also true for the brain, one could predict that there would be decreased production of NO and increased activation of the JAK-STAT pathway, both factors could influence the inflammatory state of the brain.

4. Inflammation Hypothesis and Future Directions.

We hypothesize that the triplication of chromosome 21 as occurs in DS will result in a greatly exacerbated M1 inflammatory response. The basis for this hypothesis is the range of genes that are found on chromosome 21 and, therefore, triplicated. We have discussed each of the genes that are relevant to inflammation above and have summarized what these may mean to inflammation in Figure 2. Since most of the genes are primarily associated with the M1 inflammatory response, we predict that this is the main state that will be enhanced in the DS brain. Triplication of the major interferon receptors IFNAR1, IFNAR2, and IFNGR2 means that there will be enhanced interferon signaling. In turn, this enhanced signaling will increase production of M1 markers IL-1β, TNFα, and IL-6.While these components are known to result in oxidative stress, the triplication of TIAM1, SOD1, and PRMT2 will greatly exacerbate this oxidative stress. TIAM1 enhances oxidation by inducing NADPH oxidase, SOD1 at high concentrations has been shown to enhance oxidation, and PRMT2 inhibits nitric oxide production, which acts as an antioxidant in the brain at physiologic concentrations. All of these factors will combine to enhance neurodegeneration in the DS brain in response to primary pathologies such as amyloid plaques and neurofibrillary tangles.

In considering inflammation in DS, there is a relative lack of data relative to other disorders. While AD provides us with significant background information on the role of inflammation in the disease, it is clear that the condition of DS, and the triplication of so many inflammatory-associated genes, creates a unique inflammatory environment worthy for further study. The data obtained through the study of inflammation in DS will be essential to further not only the study of DS but also, in turn, the normal inflammatory pathways in neurodegenerative disorders.

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Review Article

Alzheimer's Disease and Vascular Deficiency: Lessons from Imaging Studies and Down Syndrome

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Down syndrome (DS) individuals are at high risk for developing Alzheimer's disease (AD) and consequently provide a unique opportunity to examine the factors leading to the onset of AD. This paper focuses on the neglected vascular parallels between AD and DS that can readily be examined in DS. Several recent AD studies provide evidence that internal jugular vein (IJV) reflux may result in white matter lesions and a 30% decrease in cerebrospinal fluid (CSF) clearance of amyloid-β. At the same time, studies analyzing the synthesis of amyloid-β in DS showed greater than expected amounts of Aβ than would be predicted by the increase in gene dosage, perhaps due to slower clearance. These studies are discussed along with the possibility that the venous and CSF dysfunction found in AD patients may be present early in life in persons with DS, leaving them particularly vulnerable to early onset AD. Studying IJV function in DS provides an opportunity to understand the role of vascular function in the initiation of AD.

1. Introduction

The brains of most individuals with Down syndrome (DS) who are over 40 years old will have sufficient neuropathology for a postmortem diagnosis of Alzheimer's disease (AD) and provide an ideal population to examine novel ideas about the causation of AD. DS is a very complex genetic disorder that produces detrimental changes to many organ systems. The mechanism(s) by which the extra copy of chromosome 21 or parts thereof produce these changes is largely unknown [1]. The majority of research and therapeutic efforts to date have focused on the diagnosis and surgical correction of major heart defects associated with DS, once a major killer of children with the condition. With the cardiovascular defects surgically corrected, the average lifespan of persons with DS has increased significantly. Consequently, the spectrum of threats to persons with DS include childhood illnesses early in life and the development of AD later in life.

Increasing evidence indicates that AD is a neurovascular disease, with macrovascular events such as heart attack and stroke causing sustained hypoxia preceding disease onset [2], although some cases of AD lack a vascular component. DS typically presents with many vascular defects that are rarely seen in the general population (Table 1).

Microvascular dysfunction also appears to play a significant role in AD onset and progression [5], and the vascular endothelium may be dysfunctional in DS. Recent studies describe severe dysfunction in the endothelial system in DS [6], including significantly lower levels of endothelial progenitor cells that are necessary for vascular regrowth and repair after injury [6]. This may result from the early occurrence of oxidative stress in DS [7], which has been linked to defects in vascular epithelium [8]. Consequently, when a patient with DS has an accident or event involving vascular injury, that vascular system will not repair itself as quickly or as effectively as a person without DS. Additionally, AD risk is

Table 1: Vascular defects in Down syndrome: birth defects and prenatal vascular findings [3, 4].

Birth defects	Prenatal vascular findings
Cardiac defects (VSD and ASD) (found in 50% of persons with DS)	Reverse flow in the ductus venosus (90% of all DS fetuses)
Intrahepatic venous anomalies	Placental hypovascularity (100%)
Pelvic vasculature malformations	Intrathoracic vascular lesions (more rare, probably leads to fetal demise)
Pulmonary vein obstruction	Umbilicoportal vascular anomalies (most common fetal defect in DS)
Aortopulmonary collateral arteries	
Anomalous aortic arch arteries	
Aberrant right subclavian artery (found in 20–40% of persons with DS)	
Moyamoya disease	
Arterial dysplasia	
Thrombosis of the venous sinuses	

Birth defects: anomalies found at birth or later in life. May be found due to symptoms, or may be found incidentally. Some can also be found via pre-natal ultrasound, such as the cardiac defects, and aberrant right subclavian artery.
Prenatal vascular findings: anomalies found via pre-natal ultrasound, either in a research or clinical setting. Many of these anomalies will resolve at birth.
VSD: ventricular septal defect; ASD: atrial septal defect.

increased by head trauma or stroke [2], and normal repair and regeneration normally decline with age.

Finally, a recent review of AD research by Humpel [9] proposes a much larger role for the vascular system as a whole in the development of AD and details the impact of chronic mild cerebrovascular dysfunction on the disease. Humpel proposes that the onset of AD is preceded by chronic exposure to the cardiovascular risks over many years, including hyperhomocysteinemia, hypercholesterolemia, and type-2 diabetes, all of which cause damage to the cerebrovascular system. Amyloid-β (Aβ) deposition may be a secondary consequence of these ongoing vascular insults. DS often presents with these risk factors, in addition to the vascular defects and endothelial dysfunction previously described [10–12]. Therefore, the DS population should figure prominently in studies on the onset and progression of AD.

Most of the research on DS and AD focuses on genes related to the production of Aβ. Genetic studies have confirmed that the amyloid-β protein precursor (AβPP) gene and genes associated with Aβ production are located on chromosome 21 [13]. Persons with AD also exhibit trisomy 21 in various cell types, such as skin fibroblasts, peripheral blood lymphocytes, and brain neurons, although the relevance of this to disease onset is yet to be established [14]. The overexpression of Aβ from the extra copy of chromosome 21 has been posited as the primary driver of AD in DS, with overproduction of Aβ being responsible for its deposition in the brain. Several recent imaging studies provide insight into the very early stages of AD and suggest that vascular changes figure prominently in the development and possibly initiation of AD, as in DS.

2. White Matter Changes in Alzheimer Disease

White matter changes have been found even in the preclinical stages of AD. Gold and colleagues [15] analyzed white matter changes in women at high risk for developing AD (those with at least one APOE4 allele and a family history of dementia) and compared them to women at low risk (no risk factors). Women at high risk of developing AD showed several patterns of white matter changes not present in healthy controls, including in the direct and indirect connections to the median temporal lobes, as measured by diffusion tensor imaging [15]. Additionally, Sanz-Arigita et al. [16] used fMRI to examine resting state brain function in persons diagnosed with mild AD as compared to healthy controls. Here, brains of persons with mild AD showed regional changes in function in the frontal lobes, including increased synchronization, and the caudal areas had decreased synchronization, which may be indirectly linked to white matter changes. Conversely the occipital and parietal lobes were unaffected. Sanz-Arigita et al. conclude that there may be a global loss of long distance connections between the frontal and caudal regions [16]. Interestingly, changes in the "presymptomatic individuals" [15] involved connectivity largely in the frontal tracts, while individuals with mild AD had more global changes involving long distance connectivity [16]. Further studies are required to determine whether these results accurately indicate the pattern of disease progression. While the two studies used different patient populations and imaging techniques, both support a model of progressive change in white matter function very early in disease progression.

3. Impaired Clearance of Amyloid-β

The studies described above provide imaging not previously available and are indicative of changes in the white matter in AD, but do not address the mechanism driving these changes. Overproduction of Aβ is thought to be the major source of damage to white matter in AD [2]. However, a recent study by Mawuenyega et al. provides an alternative possibility for the accumulation of Aβ in the brain [17]. In this study, the researchers used mass spectroscopy to

longitudinally measure the level of Aβ in cerebrospinal fluid (CSF), as well as the clearance and production rates of Aβ. Importantly, it should be noted that CSF clearance is largely through the white matter and is negligible in gray matter [18]. Mawuenyega et al. measured clearance and production rates for Aβ_{42} and Aβ_{40} for 36 hours in 12 patients with late-onset AD, as compared to healthy controls. The AD group had a 30% slower Aβ clearance rate than the controls, although no difference in average production rates was seen between the AD group and healthy controls [17].

CSF clearance may be an important disease marker in AD. Ott and colleagues examined increased ventricular volume as a biomarker for impaired CSF clearance [19]. They studied the relationship between ventricular volume and the AD-related biomarkers Aβ, tau, and phosphorylated tau in controls, individuals with mild cognitive impairment, and individuals with AD, taking ApoE genotype into account. Here, ventricular volume was inversely related to Aβ levels for ApoE4 controls and to tau levels in AD patients [19], although the mechanism underlying the ApoE4 effect on ventricular volume is unclear. Wastyn et al. described the protective effects of daily consumption of caffeine with regard to AD [20], which appears to be due to caffeine's affect on the CSF system and resulting clearance of various toxins, including Aβ and tau [20].

Taken together, these studies indicate that the onset of AD may not be due to an overproduction of Aβ, but rather by impaired CSF drainage and flow. Therefore, further studies are needed to determine the mechanism governing CSF drainage and flow. CSF is produced in the choroid plexus and is reabsorbed into the bloodstream via the arachnoid villi and venous sinuses [21]. Changes in CSF production, pressure, and flow rates are affected by external forces causing inflammation or leakage, including traumatic brain injury, infection, tumors, or lumbar punctures [21]. The result of impaired clearance may be specific to AD, reflecting the extracellular presence of Aβ in contrast to tau, alpha synuclein, and ubiquitin, and suggests that the role of Aβ in disease initiation and progression results from production/secretion of Aβ rather than release of Aβ following cell death.

The CSF system may also be influenced by changes in vascular flow and pressure of the venous systems near the brain. Alteration of homeostasis between the CSF and the vascular system may play an important role in the development of AD; dysfunction in the vascular systems involved with CSF may decrease CSF clearance from the brain, thereby increasing Aβ in the brain resulting in disease onset. One such age-related change in the vascular system is jugular venous reflux, which can lead to decreased cerebral perfusion pressure [22]. The internal jugular vein provides the majority of the drainage pathway for cerebral venous drainage. Jugular venous reflux results from pressure beyond the competence of the IJV valves and consequent increased backpressure limiting cerebral perfusion pressure. Jugular venous reflux is linked to a variety of other neurological disorders, including transient global amnesia, transient monocular blindness, multiple sclerosis, exertional headaches, and idiopathic intracranial hypertension [22], all of which may be linked to increased oxidative stress.

4. Internal Jugular Reflux Increases with Age

Vascular events are a prominent risk factor for the development of AD [2]. Chung and colleagues performed color-coded duplex sonography on the internal jugular veins (IJVs) of 349 subjects ranging in age from 55.6 to 89 years old [23]. These subjects comprised a large, healthy population, with age being the main variable among them. Overall IJV function changed with increasing age, although this occurred particularly in the left IJV, including increased lumen area, increased jugular venous reflux, and slower velocity. These findings are consistent with decreased left IJV outflow with aging [23].

5. White Matter Changes with Jugular Reflux

Changes in white matter often occur with the onset of AD [15, 16]. In a recent MRI and ultrasound study, white matter changes were also found to be associated with IJV reflux [24]. Here, MRI and ultrasound were used to analyze the brains and IJVs, respectively, of 97 individuals ranging in age from 55 to 90 years old. The ultrasound results were grouped into three categories of jugular venous reflux: none, mild, and severe. Persons with severe jugular venous reflux had more white matter changes than either the mild or no reflux groups, particularly in caudal brain regions. Further, whole brain white matter changes were more prominent in persons greater than 75 years old that had severe venous reflux [24], consistent with previous findings.

Taken together, these studies provide a potential mechanism by which IJV function affects CSF flow, leading to the development of AD. Specifically, IJV function declines with age, resulting in reflux, slower velocity, and decreased venous outflow. This decreased flow produces changes in venous pressures, which then alters pressure in the CSF system. The CSF system decreases outflow from the brain to restore homeostatic pressure in the brain. As a consequence, Aβ begins to accumulate within the brain instead of being cleared via the CSF. Increased Aβ accumulations lead to damage to white matter, beginning with the temporal lobes and expanding to frontal and caudal regions, perhaps ultimately leading to clinical features associated with AD.

6. Relevance to Down Syndrome

The CSF clearance study by Mawuenyega et al. [17] provides evidence that, in the general population, clearance may be more important in the etiology of AD than is production of Aβ. Currently, no comparable studies in a DS population have been conducted, although several studies provide indirect evidence that Aβ clearance may be a factor in DS. Gyure and colleagues found that serum levels of Aβ are 200–300% higher in DS individuals as compared to controls [25], possibly due to overproduction of Aβ. Wolvetang and coworkers examined Aβ production in relation to the predicted effect of

gene dosage and found that Aβ expression is 3-4 times higher in DS individuals, rather than 1.5 times higher as would be predicted due to the extra chromosome 21. Wolvetang et al. concluded that an additional transcriptional regulator of Aβ also located on chromosome 21 may cause overexpression of Aβ protein in DS [26]. Finally, Choj et al. examined levels of AβPP with increasing age in a mouse model of DS [27] and determined that DS mice expressed the same level of AβPP as controls at 4 months of age. However, by 10 months of age, the DS mice exhibited increased levels of AβPP [27], although Aβ_{40} and Aβ_{42} were not increased. Choj et al. concluded that the changes in AβPP levels are due to "multiple mechanisms of regulation" [27]. Taken together, overproduction alone could not fully explain increased Aβ levels. As reviewed in Wiseman et al. [28], a number of additional genes on chromosome 21 have been implicated in the development of AD in DS individuals, including those involved in tau hyperphosphorylation (e.g., DYRK1A).

7. Down Syndrome and Alzheimer Disease: Vascular Risks

Given the large number of known vascular problems present in individuals with DS, it is possible that the vascular system associated with CSF clearance, particularly the IJVs studied by Chung et al. [23, 24], could be impaired in DS and that this impairment would likely begin early in life. Chronic, yet mild, dysfunction of the IJV beginning early in life, along with resulting impairment in CSF clearance, would leave persons with DS particularly vulnerable to the buildup of Aβ in the brain, which may be exacerbated by the overproduction of AβPP due to increased gene dosage.

Together, the studies described above addressed very specific questions relating to gene expression and protein production, but did not examine CSF clearance. Based on the CSF clearance study, one should question whether increased production of Aβ is the only cause of high levels of Aβ in DS. Perhaps persons with DS have severe CSF clearance issues along with increased production. Or could it be due to complications from cardiovascular problems seen in early life and not fully corrected by surgical treatment?

The role of IJV reflux and CSF clearance of Aβ in the development of AD in the DS population is currently unknown. Determining whether these two conditions occur in the DS population would help to clarify the role of overproduction of Aβ versus the role of vascular defects and dysfunction in the development of AD for persons with DS.

Replicating the studies described above in a DS population would provide answers to several key questions, including the following.

CSF Clearance of Aβ in Down Syndrome.

(i) Is the rate of Aβ production increased in DS relative to healthy controls, to AD patients?

(ii) Do adult patients with DS and AD exhibit decreased CSF clearance of Aβ?

(iii) Do adult patients with DS, but not AD, exhibit decreased CSF clearance of Aβ?

(iv) Do children with DS exhibit decreased CSF clearance of Aβ?

IJV Function and Resulting White Matter Changes in Down Syndrome.

(i) How do the IJVs function in adults with DS, as compared to healthy controls? Compared to those with AD?

(ii) Does IJV function deteriorate more quickly in DS than in healthy controls and/or those with AD?

(iii) Do persons with DS present with jugular reflux? If so, do they also present with changes in white matter consistent with the patients previously studied?

(iv) Do children with DS present with IJV dysfunction. That is, at what age does jugular reflux begin?

Taken together, the studies outlined above suggest a temporal sequence of events beginning with increased oxidative stress, an early feature of AD. Chronic oxidative stress, in turn, may lead to decreased vascular function and ultimately results in increased Aβ deposition. The increased expression of AβPP and Aβ appears to be a compensatory response to stress and deposition may simply reflect the failure of this response to alleviate chronic stress in the context of decreased clearance. Studies of the DS population will aid in clarifying these interactions, perhaps elucidating a potential point of intervention in the development of AD pathology in these individuals.

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Review Article

Oxidative Stress and Down Syndrome: A Route toward Alzheimer-Like Dementia

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Down syndrome (DS) is one of the most frequent genetic abnormalities characterized by multiple pathological phenotypes. Indeed, currently life expectancy and quality of life for DS patients have improved, although with increasing age pathological dysfunctions are exacerbated and intellectual disability may lead to the development of Alzheimer's type dementia (AD). The neuropathology of DS is complex and includes the development of AD by middle age, altered free radical metabolism, and impaired mitochondrial function, both of which contribute to neuronal degeneration. Understanding the molecular basis that drives the development of AD is an intense field of research. Our laboratories are interested in understanding the role of oxidative stress as link between DS and AD. This review examines the current literature that showed oxidative damage in DS by identifying putative molecular pathways that play a central role in the neurodegenerative processes. In addition, considering the role of mitochondrial dysfunction in neurodegenerative phenomena, results demonstrating the involvement of impaired mitochondria in DS pathology could contribute a direct link between normal aging and development of AD-like dementia in DS patients.

1. Down Syndrome

Down syndrome (DS) is the most common genetic cause of mental retardation that arises from the triplication of the entire, or even part of chromosome 21 (trisomy21). Although the genetic alterations are responsible of the major clinical presentations of the disease such as craniofacial abnormalities, small brain size, accelerated aging, and cognitive defects, additional environmental factors seem to play an important role in determining the severity of multiple phenotypes. Genetic instability due to trisomy leads to the development of two types of phenotypes: (1) those present in every DS individual and (2) those that occur only in a subset of DS individuals. In addition, for any given phenotype there is considerable variability in expression that further results in a complex, "not-predictable" set of clinical signs [1]. For example, the extent of cognitive impairment in the DS population presents with a wide range of diversity. This wide variability may be explained, at least in part, by the "gene dosage hypothesis," which states that some of the genes encoded on Chr21 are dosage sensitive—that is, three copies result in phenotypic effects—and contribute to the phenotypes of DS [2, 3]. This proposed scenario is further complicated by the fact that the abnormal expression of trisomic genes also affects disomic genes as well, which, in turn, are in part responsible of some clinical manifestations and ultimately results in as assembly of different DS phenotypes [4]. Thus, according to "the amplified developmental instability hypothesis", the most important cause of the array of phenotypic features does not actually involve exclusively specific genes on Chr21 but rather elevated activity of sets of genes, regardless of their identity, which lead to a decrease in genetic stability or homeostasis [5] An interesting example of this effect is represented by the findings that newborns and children with DS are predisposed to a range of blood disorders, which include acute lymphoblastic leukaemia

and acute megakaryocytic leukaemia (AMKL). In addition to trisomy 21, fetal haemopoietic progenitors acquire N-terminal truncating mutations in the key megakaryocyte-erythroid transcription factor GATA1 [6].

Improvements in quality of life of individuals with DS have resulted from improvements in medical care, identification and treatment of psychiatric disorders (such as depression, disruptive behavior disorders, and autism), and early educational interventions with support in typical educational settings [7, 8]. Also, largely owing to advances in medical care and attitude changes, the median age of death in this population has increased to 49 years, and the life expectancy of a 1-year-old person with Down syndrome is more than 60 years and is likely to improve [8].

Considering that DS patients presently have an improved life expectancy and quality of life, the comprehension of degenerative phenomena related to accelerated aging and neurodegeneration has received much attention from researchers. In fact, a link between the DS phenotype and an increased risk of the development of AD has now been firmly established [9]. The prevalence of dementia among DS patients is 8% in the age range 35–49, 55% in the age range 50–59, and 75% above the age of 60 years, but AD neuropathology is present in all of the cases by the age of 40 [10]. AD like dementia in DS population is characterized by the presence of senile plaques (SPs) and neurofibrillary tangles (NFTs) and by cholinergic and serotonergic reduction [11, 12]. However, although most DS patients have plaques early in life and even in the fetus, it is only very later on that they may develop AD. Thus, identification of common pathways together with specific differences of the neurodegenerative process occurring both in DS and AD currently represents an intense field of research. Among proposed hypothesis, oxidative stress is receiving much attention and may be considered a bridge between DS and AD.

2. Oxidative Stress (OS) in DS

Increasing number of studies have recently shown that OS occurs in DS pathogenesis and progression due to a deregulation of gene/protein expression associated with the trisomy characteristic of DS [13]. Increased production of ROS is also accompanied by mitochondrial dysfunction, which occurs in DS cells as early as from embryonic life [14]. Although oxidative stress implications in DS phenotype have been demonstrated [10, 11, 15–17], a direct cause-and-effect relationship between the accumulation of oxidatively mediated damage and clinical manifestation of DS is not yet strongly established. Growing evidence supports the occurrence of chronic oxidative injury in the brain that could imply a risk factor for subsequent neurodegeneration in aged DS patients [4, 18, 19]. Increased conditions of oxidative stress are caused by the overexpression of some of the genes encoded by Chr21 (Figure 1). Among these, one of the most relevant as a potential OS inducer is copper-zinc superoxide dismutase (SOD1). SOD1 is thought to have a major role in the first line of antioxidant defense by catalyzing the dismutation of $O_2^{\bullet -}$ to molecular oxygen (O_2) and H_2O_2, which can be converted by catalase (CAT) and by (selenium-containing) glutathione peroxidase (GPX) to water [20]. The triplication of Chr21, on which the SOD-1 gene is localized, leads to an imbalance in the ratio of SOD-1 to CAT and GPX, resulting in the accumulation of H_2O_2 [10]. Interestingly, all DS tissues, in addition to the brain, display an altered SOD-1/GPX activity ratio [21]. SOD-1 was found at levels approximately 50% higher than normal in a variety of DS cells and tissues, including erythrocytes, B and T lymphocytes, and fibroblasts. Indeed, the erythrocytes of DS children, adolescents, and adults exhibited systemic increases in SOD-1, SOD-1/GPX, or the SOD-1/(GPX + CAT) activity ratio. In addition, a decreased expression of peroxiredoxin 2 was detected in DS fetal brain which may contribute to enhanced susceptibility of DS neurons to free radical attack [22].

A crucial role of SOD-1 is further demonstrated by the study of Shin et al. which reported that transgenic mouse strains overexpressing wild-type human SOD1 (Tg-SOD1) showed to have mitochondrial swelling, vacuolization, or learning and memory deficits [23]. Mitochondrial ATP synthase alpha/beta chain and elongation factor Tu were aberrant in Tg-SOD1, while antioxidant proteins were found to be unchanged. Derangement of neuronal and mitochondrial proteins may indicate synaptosomal and neuronal loss in Tg-SOD1 hippocampus, already reported in morphological terms, and could help to understanding brain deficits in DS.

Consonant with the above-cited studies, Busciglio and Yankner [14] reported that neurons of DS patients exhibited a sharp increase in intracellular ROS which is also accompanied by elevated levels of lipid peroxidation. In addition, a proteomics study from Gulesserian et al. [24] showed that oxidative stress in fetal DS did not result from overexpression of SOD-1 protein but appeared to be the consequence of low levels of antioxidant enzymes involved in removal of hydrogen peroxide, such as glutathione transferases and thioredoxin peroxidases.

Interestingly, elevated levels of OS could also be caused by increased release of amyloid beta-peptide (Aβ). Many studies demonstrated that both Aβ(1-40/42) are able to induce OS [25–31]. Thus, the overexpression of the amyloid precursor protein (*APP*) gene, which is also encoded by Chr21, could explain in DS patients the overproduction of Aβ peptide, the major protein in SPs. Indeed, postmortem studies on DS brain evidenced accumulation of Aβ(1-42) peptide, a characteristic hallmark of AD pathology, which correlate with age [32]. Mehta et al. [33] found that Aβ(1-42) and Aβ(1-40) levels were higher in DS plasma than controls. The ratio of Aβ42/Aβ40 was lower in DS than in controls and a significant negative correlation between age and Aβ40 in DS and controls were observed, and between age and Aβ42 levels in DS but not in controls. Recently, a paper from the same group demonstrated that among adults with DS, decreasing levels of plasma Aβ42, a decline in the Aβ42/Aβ40 ratio, or increasing levels of Aβ40 may be sensitive indicators of conversion to AD, possibly reflecting compartmentalization of Aβ peptides in the brain [34]. However, recent studies from Anandatheerthavarada et al.

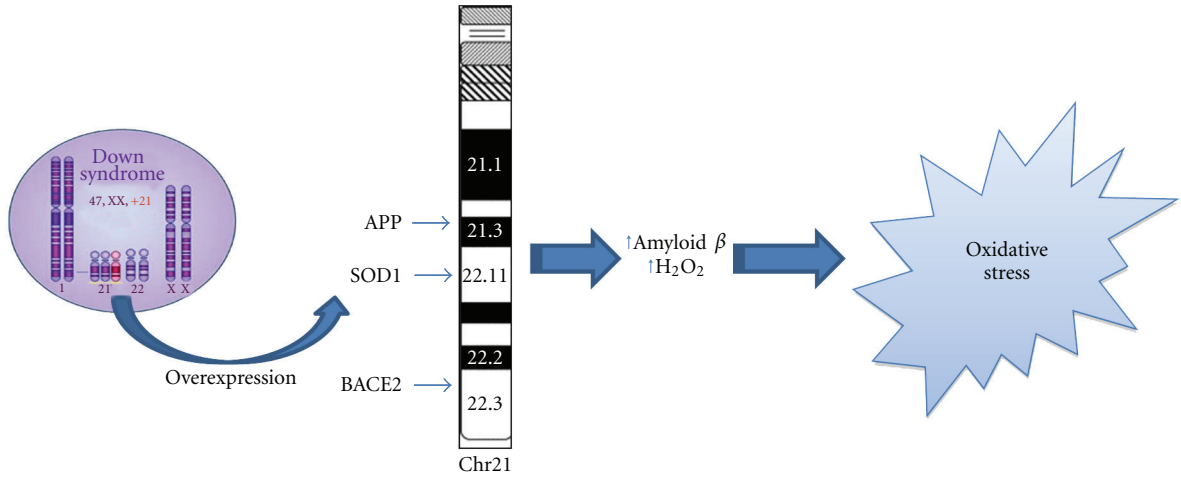

Figure 1: Oxidative stress and down syndrome. Increased conditions of oxidative stress are caused by the overexpression of some of the genes encoded by Chr21. Among these, amyloid precursor protein (APP), copper-zinc superoxide dismutase (SOD1), and beta secretase (BACE2) can directly or indirectly lead to OS.

[35] indicated that also full length APP itself may have deleterious effects, particularly targeting mitochondria. The authors proposed that under increased APP expression, a progressive accumulation of transmembrane-arrested APP caused perturbation of mitochondrial function, which in turn resulted in impairment of energy metabolism.

Moreover, mice overexpressing wild type human APP display cognitive defects and neuronal pathology similar to AD; these mice do not show significant Aβ deposition in the hippocampus [36]. In these mice, hAPP processing was basically nonamyloidogenic, while increased levels of phosphorylated tau in the hippocampus were observed. These findings support the notion that trisomy of APP may promote mitochondrial dysfunction in DS independent of aberrant Aβ deposition.

Related to APP metabolism, another gene encoded by Chr21 is the β-site APP-cleaving 2 enzyme (BACE2). BACE is homologous to BACE1, a β-secretase involved in the amyloidogenic pathway of APP proteolysis, and thus it has been hypothesized that the co-overexpression of both genes could contribute to Alzheimer's like neuropathology present in DS. However, co-overexpression of BACE2 and APP did not increase amyloid-β peptide concentration in brain of Tg mice. These results suggest that the in vivo effects of APP are not exacerbated by BACE2 co-overexpression but may have some protective effects in specific behavioral and cognitive domains in transgenic mice [37].

By mapping Chr21, another candidate gene that may be involved in OS is the enzyme carbonyl reductase (CBR). Carbonyls, which are cytotoxic metabolic intermediates, are detoxified by either oxidation catalyzed by aldehyde dehydrogenase (ALDH) or by reduction to their corresponding alcohols by carbonyl reductase (CBR) and/or alcohol dehydrogenase (ADH). Protein levels of both enzymes were found to be increased in several brain regions of both DS and AD patients because of enzyme induction by elevated carbonyls in DS and AD [38]. Further, carbonyl reductase is an oxidatively modified protein in brain of subjects arguably with the earliest form of AD, mild cognitive impairment [39].

There is evidence of a link between 1-carbon/trans sulfuration (1C-TS) metabolism and DS. There are at least six genes encoding enzymes important for 1C-TS metabolism located on human Chr21, including the gene for cystathionine beta synthase (CBS) [40]. CBS catalyzes the condensation of serine and homocysteine to form cystathionine. It plays a critical role in linking the folate cycle and the methionine cycle and in regulating homocysteine levels [41]. In addition, CBS can convert cysteine to hydrogen sulfide, which researchers are beginning to recognize as an important neuromodulator in the brain [42]. There is evidence that CBS protein levels and enzyme activity are increased in persons with DS [43]. Elevated CBS activity can lower homocysteine levels, which in turn perturb the balance of 1C-TS metabolism and lead to elevated—perhaps toxic—levels of hydrogen sulfide. These metabolic alterations might play a role in the cognitive disability seen in DS [44, 45]. Accordingly, CBS is considered a risk factor for AD [46].

Another player in the complex C1-TS metabolism is also the trifunctional enzyme complex glycinamide ribonucleotide synthase-aminoimidazole ribonucleotide synthase-glycinamide formyl transferase (GARS-AIRS-GART), which catalyzes certain steps of *de novo* purine synthesis [47]. GART is aberrantly regulated and overexpressed in DS individuals and may be involved in the phenotype of DS [47]. Accumulation of uric acid, the end product of purine metabolism, is another feature of DS and there are some hypotheses about the pathogenetic mechanism leading to its increase [48]. Hyperuricemia has an interesting relationship with oxidative stress since it represents an important free radical scavenger and ROS themselves could influence its increase.

Chr21 also maps the gene for S100β, an astroglial-derived Ca^{2+}-binding protein having neurotrophic role on neurons and glial cells. S100β is responsible to start up a gliotic reaction by the release of proinflammatory mediators, including nitric oxide and cytokines from microglia and astrocytes, which are, in turn, deleterious for neurons [49]. Interestingly, proinflammatory effect of S100β seems not

to be restricted into the brain. Macrophages play a pivotal role in inflammatory diseases, occurring both in the brain and in the periphery. An aberrant S100β production has been observed in DS and AD [50]. It has been shown that S100β stimulates both NO production and iNOS protein transcription and expression in rat peritoneal macrophages [49].

Elevated OS has been demonstrated in peripheral and CNS specimens of DS patients and models thereof [14]. Increased levels of TBARS, total protein carbonyls, and advanced glycation endproducts (AGEs) in the cortex from DS fetal brain compared with controls were reported [51] and a marked accumulation of 8-hydroxy-2-deoxyguanosine (8OHdG), oxidized proteins and nitrotyrosine, in the cytoplasm of cerebral neurons in DS was found [52]. Elevated levels of isoprostane 8,12-*iso*-iPF2α (iPF2α), a specific marker of lipid peroxidation, have been measured in urine samples from adults with DS [25]. In addition, levels of AGEs, dityrosine, H_2O_2, and nitrite/nitrate were found to be significantly increased in urine samples of DS compared with age-matched controls [53]. These markers of oxidative damage were considered more consistent compared with 8-OHG, 15-F(2t)-IsoP, and TBARS which gave contrasting results. However, additional studies on large population are needed to confirm the reproducibility of these results.

The majority of OS data have been obtained by the analysis of animal models of the disease, including Ts65Dn mice and Ts1Cje mice. The Ts65Dn mouse carries a small chromosome derived primarily from mouse chromosome 16, causing dosage imbalance for approximately half of human chromosome 21 orthologs. These mice have cerebellar pathology with direct parallels to DS [54]. The Ts1Cje mouse, containing a translocated chromosome 16, is at dosage imbalance for 67% of the genes triplicated in Ts65Dn [55]. Ts1Cje mice do not express the SOD1 gene and show some DS-related abnormalities such as craniofacial alterations [56] and spatial learning deficits [57], but different from Ts65Dn mice.

Ishihara et al. [27] reported increased level of ROS and mitochondrial dysfunction in primary cultured astrocytes and neurons from Ts1Cje transgenic mice, confirming that the "gene-dosage" hypothesis is sufficient to explain, at least, the major part, of OS-induced intracellular damage observed in this animal model of DS. The authors also identified by redox proteomics approach the putative target proteins that were modified by lipid peroxidation-derived products [27]. ATP synthase mitochondrial F1 complex b subunit, α-enolase, and triosephosphate isomerase 1 were identified as proteins modified by 3-hydroperoxy-9Z,11E-octadecadienoic acid (13-HPODE). Neurofilament light polypeptide, internexin neuronal intermediate filament α, neuron specific enolase, peroxiredoxin 6, phosphoglycerate kinase 1, and triosephosphate isomerase were shown to be HNE-modified proteins. Thus, dysfunction of these proteins as a consequence of oxidative damage may affect ATP production, the neuronal cytoskeleton system, and antioxidant network function. Interestingly, previous redox proteomics studies from our laboratory previously found some of these proteins modified by hydroxynonenal, a reactive product of lipid peroxidation, in AD and MCI brain [39, 58, 59], suggesting that these brain proteins might contribute to cognitive dysfunction and neurodegenerative processes occurring in DS. These findings point out that DS and AD share common pathways of neurodegeneration that need to be further elucidated.

In an effort to better understand the role of oxidative stress we have analyzed the amniotic fluid (AF) from women carrying DS pregnancy compared with that from women carrying healthy fetuses. While the majority of the studies have been performed on Down fetal brains or DS mouse model, few data are available on AF, which is a more reliable index of the physiological condition of the fetus. In analogy with CSF, which is considered "a window into the brain", AF could be used for the identification of disease biomarkers to be coupled with current genetic screening. Thus, AF provides both physical and biochemical support for the developing fetus. Its composition is complex and includes fetal and maternal proteins, amino acids, carbohydrates, hormones, lipids, and electrolytes. Since AF is in direct contact with multiple organs of the fetus, AF contains high concentrations of proteins that are directly secreted from the fetus [60]. Not surprisingly, recent technological advances in proteomics have been actively utilized to investigate AF, in order to better understand its complex biological function and to discover disease-specific biomarkers for fetal aneuploidies and pregnancy-related complications. Once an abnormal proteomic profile is identified, it has to be compared with healthy closely matched controls, allowing for a disease-specific biomarker to be identified [61, 62].

Thus, we have evaluated a set of oxidative stress biomarkers in amniotic fluid from women carrying DS fetuses, and we found increased levels of oxidative stress, as indexed by increased protein oxidation, lipid peroxidation, reduction of GSH and Trx levels, and induction of the heat shock protein (HSP) response. By a redox proteomics approach, we have identified selective proteins that showed increased oxidation in DS AF compared with that from mothers carrying healthy fetuses. The identified proteins are involved in iron homeostasis (ceruloplasmin and transferin), lipid metabolism (Zinc-alpha2-glycoprotein, retinol binding protein 4 and Apolipoprotein A1), and inflammation (Complement C9, Alpha-1B-glycoprotein, Collagen alpha-1 V chain) with critical relevance in the clinical outcome of DS [63].

As previously mentioned, another important player in the oxidative stress hypothesis of neurodegeneration is Aβ peptide. Brain from DS subjects show consistent Aβ deposition and neurofibrillary tangle formation [64] that correlate with of age. Although plaque deposition is a very early event in DS patients, even in fetal development, it is only very later on that they may develop AD [65]. In fact, increased signs of dementia in DS after the age of 50 years appeared many years later the first signs of significant insoluble Aβ accumulation or plaque deposition and also after the first signs of neurofibrillary tangle pathology [66]. Thus, other factors, which may not directly link Aβ metabolism and tangles formation, have to be involved to cause consistent neuronal dysfunction and cognitive decline. Synaptic dysfunction may be a consequence of APP overexpression or increased Aβ

[67]. Neuroinflammation [68], endosomal dysfunction [69], and oxidative damage [52] may play a crucial role in DS as well as in AD pathology [66].

Recent studies by our laboratory [17] were performed to establish an association between brain oxidative damage and Aβ neuropathology as a function of age in DS patients. Preliminary results showed that DS brains with neuropathological hallmarks of AD have more oxidative, but not nitrosative, stress than those with DS but without significant AD pathology, as compared with similarly aged-matched non-DS controls. Further studies are needed to better understand aging-related phenomena in DS, which from one side contribute to development of AD but also paradoxically result in AD neuropathology but without dementia.

The neuropathology of DS is complex and occurs with a wide variability. The characteristic hallmarks of neurodegenerative process are altered free radical metabolism and impaired mitochondrial function which both contribute to development of AD by middle age [70–72]. However, recent studies reported a quite surprising trend of oxidative stress damage in DS. While increased OS is detectable as early as during pregnancy [73] and increases over age in young DS, adults with DS do not show a significant increased oxidative damage to DNA [74]. These data could appear contradictory with other findings supporting the correlation of increased oxidative damage with increasing age. One of the reasons could be related to the samples analyzed, that is, peripheral lymphocytes, which indicate a cell-type with specific functionality and which could be able to activate compensatory mechanisms as the brain does not. In addition, different markers of oxidative stress do not always correlate with each other, because the ability of the cell to repair differently a "specific" damage in addition to different susceptibility of lipids, proteins, and nucleic acid to accumulates oxidative damage.

It seems likely that young DS experienced a sort of chronic oxidative stress and those "surviving" cells become more resistant by activating defense mechanisms that counteract increasing oxidative stress conditions over the lifespan [74]. This is reasonable by considering that newborn DS have to challenge with high levels of ROS that are responsible of the pathogenesis of many of the pathological manifestations. In contrast, this "experience" of OS promotes the survival of more resistant cellular phenotypes that show several dysfunctions (Figure 2). This hypothesis is further supported by studies from Head et al. [65], who showed by PET that compensatory increases in metabolic rate and activation of plasticity mechanisms in vulnerable brain regions in DS occurred prior to the development of dementia. The same genes, including APP, DYRK1A, SOD1, and RCAN1, which once overexpressed are responsible of impaired neuronal growth and synapse maintenance on the contrary may also promote the activation of compensatory mechanisms during aging [65].

For example, secreted forms of AβPP, in addition to be neurotoxic, can also function as neuroprotective factors [75] and possible cell adhesion molecules [76] and also play a role in cell signaling [77]. Interaction of AβPP with multiple protein networks might result in activation of complex compensatory responses. RCAN1 (regulator of calcineurin 1) has recently been shown to act synergistically with DYRK1A to impair the function of NFAT transcription factors which are involved in cell development. RCAN1 is highly expressed in neurons and overexpressed in DS brain [78]. Possible additional roles for RCAN1 include modulation of the chromosome 21 gene SOD1 [79] and playing a critical role in mitochondrial function [80]. It seems likely that some trisomic genes may interact with each other and are responsible of learning and memory deficit during development, but with increasing age their interaction may become beneficial and possibly protective [60]. The molecular mechanisms which drive dysfunction versus protection need to be extensively investigated. Based on these considerations, enhancing or supporting compensatory mechanisms in aging individuals with DS may be beneficial as suggested by intervention studies in animal models.

3. Mitochondrial Dysfunction in DS

Several reports have demonstrated that mitochondrial impairment plays a central role on neurodegeneration [16]. The first abnormalities of mitochondrial function (abnormal shape, reduced levels of microtubules, etc.) was observed in cultured cerebellar neurons from trisomy 16 (Ts16) mice [81]. Previous findings demonstrated deficient functionality of mitochondrial enzymes, including monoamine oxidase, cytochrome oxidase, and isocitrate dehydrogenase [82].

Numerous studies have demonstrated that the accumulation of mitochondrial DNA (mtDNA) mutations is a major contributor to degenerative diseases and human ageing [83]. Studies from several groups suggested that mtDNA mutations have a role in the pathogenesis of DS [71, 84, 85]. Apart from helping to explain free radical damage and development of AD, the presence of mtDNA mutations could explain the association of DS with premature ageing and diabetes [86]. Mutations in mtDNA may bring about an increase in the generation of free radicals and reduce ATP levels. This, in turn, could affect the synaptonemal complex and chromosome segregation, also alter recombination and so lead to aneuploidy.

Druzhyna et al. [87] demonstrated not only an increase of mtDNA oxidative damage but also a reduced functionality of specific repair systems in fibroblasts from DS patients. Increased oxidative damage was a consequence of increased superoxide formation that was demonstrated in Ts16 neurons compared to control neurons. This condition persisted also in the presence of rotenone, a mitochondrial respiratory chain complex I inhibitor, which was able to block $O_2^{\bullet -}$ production in diploid neurons, but not in Ts16 neurons. This different behavior between Ts16 neurons and diploid neurons also was evident when cells were treated with carbonyl cyanide p-trifluoromethoxyphenylhydrazone, used to uncouple mitochondrial oxidative phosphorylation, which caused irreversible deficiency in the energy metabolism in Ts16 neurons, but not in diploid control neurons. Thus, an

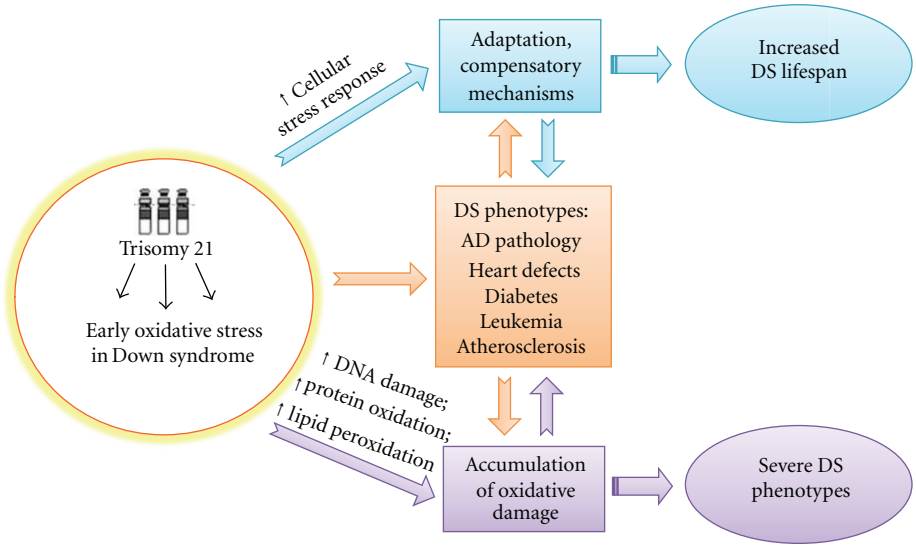

Figure 2: Putative adaptation to OS in down syndrome. OS occurs early in DS pathogenesis and progression. Accumulation of oxidative damage leads to severe phenotypes while the induction of compensatory mechanisms in response to chronic OS could result in "adaptation" and could contribute to improve the life span of DS subjects.

increased $O_2^{\bullet -}$ basal generation in Ts16 neurons results from deficient complex I coupled with an impaired mitochondrial energy metabolism that ultimately leads to neuronal cell death [88]. A selective impairment of complex I activity was demonstrated in isolated cortex mitochondria from Ts16 mice by administration of its normal substrates, malate, and glutamate, but not with the Complex II substrate succinate [89]. Accordingly, a very recent paper by Valenti et al. [90] reported a selective deficit in the catalytic efficiency of Complex I in DS-HSFs (Down's syndrome human fetal skin fibroblasts). The Complex I deficit was associated with a decrease in cAMP-dependent phosphorylation of the 18 kDa subunit of the complex, due to a decrease in PKA (protein kinase A) activity related to reduced basal levels of cAMP. Furthermore, the authors measured a 3-fold increase in cellular levels of ROS, in particular $O_2^{\bullet -}$, mainly produced by DS-HSF mitochondria. This effect was prevented by dibutyryl-cAMP, a membrane-permeable cAMP analogue, suggesting its involvement in ROS production.

H_2O_2 production and calcium uptake did not differ significantly in the Ts16 mitochondria, while a decrease in pyruvate dehydrogenase levels was detected, similar to the pattern found in Parkinson's disease [91].

Further details on mitochondrial functionality were underscored by a study by Conti et al. [13]. The authors analyzed the expression profile of several genes located on Chr21 using oligonucleotide microarrays in hearts of human DS fetuses compared with normal fetuses. The authors concluded that dosage-dependent upregulation of Chr21 genes causes dysregulation of the genes responsible for mitochondrial function and for the extracellular matrix organization in the fetal heart of trisomic subjects [13].

Direct evidence for an in vivo alteration of mitochondrial function in blood cells from DS patients was reported by Roat et al. [92], who found an increased loss of $\Delta\Psi(m)$, underlying the presence of an increasing susceptibility of these organelles to damaging agents. As noted above, mitochondrial function is also regulated by the methyl status due to the presence on Chr21 of the gens for specific CBS, which participates in recycling of methionine/homocysteine in the methyl cycle sequence of reactions. In fact, methylation is a necessary event in mitochondria and relies on the availability and uptake of the methyl donor S-adenosylmethionine. Indeed mitochondrial dysfunctions have been widely described in DS, but they have never been correlated to a possible mitochondrial methyl unbalance. Infantino et al. [93] recently showed that the mitochondrial levels of S-adenosylmethionine were reduced in DS compared to control cells consistent with a methyl imbalance on mitochondria functionality.

4. Concluding Remarks

Within the context of the reported findings discussed above, we hypothesize that trisomy affects gene/protein expression that results in increased OS conditions and impaired mitochondrial function. These alterations occur early in DS as demonstrated by studies performed on fetal brain and amniotic fluid from DS pregnancy and play an important role in neurodegeneration.

Several studies suggested different mechanistic causes for the changes in redox state in contributing to early neural pathological changes in DS brain. OS conditions arise not only from overexpression of SOD1 but also as a consequence of low levels of reducing agents and antioxidant enzymes. Redox imbalance is further affected by overproduction of Aβ, which accumulates into plaques across the lifespan in DS as well as in AD. Aβ toxicity has been shown to be a major effector of neuronal loss and cognitive dysfunctions observed both in DS and in AD and contributes to exacerbate oxidative damage into the brain. In fact, OS is a crucial factor because it affects multiple pathways related to cell growth/death, gene

expression, and protein function, among many others. It is now well accepted that OS contribute to neurodegeneration, but in the case of DS and AD, genetic similarities, due to the fact that some of the genes responsible for familial form of AD are encoded by Chr21, provide an interesting field of research for the comprehension of many yet unsolved issues.

Based on this notion, it is possible that using antioxidant nutrients to scavenge oxygen-derived free radicals may modulate some of the complications of DS. A very recent paper by Lott et al. [94] demonstrated that a 2-year randomized, double-blind, placebo-controlled trial with daily oral antioxidant supplementation (900 IU of alpha-tocopherol, 200 mg of ascorbic acid, and 600 mg of alpha-lipoic acid) was effective, safe, and tolerable for individuals with DS and dementia. However, individuals receiving the antioxidant supplement showed neither an improvement in cognitive functioning nor a stabilization of cognitive decline compared with control group.

These data are in contrast with those obtained by Lockrow et al. [95], who treated Ts65Dn mice with a long-term antioxidant supplementation. Supplementation with vitamin E effectively reduced the levels of ROS in the adult Ts65Dn brain. Also, Ts65Dn mice receiving vitamin E exhibited improved performance on a spatial working memory task and showed an attenuation of cholinergic neuron pathology in the basal forebrain.

This discrepancy likely results from the "biological gap" between human and animal studies. Though transgenic mice are a useful model to study the molecular basis of a disease and test the efficacy of drug treatment, they do not show all the features of human disease. In particular, when testing the protective effects of antioxidants, supplementation has to be initiated before persistent oxidative damage occurs. For example, many individuals with AD most likely have significant AD pathology by the time of diagnosis. This phenomenon, reasonably, is one of the major limits of clinical trials that should be taken into account, such that antioxidants should be administered as putative modulators of disease at the very early stage of the disease.

Although limits of antioxidant therapies exist, an intriguing prospective could be offered by the comprehension of putative compensatory mechanisms which are activated even in the presence of genetic instability in the DS population that could play a role in explaining the wide variability of phenotypes. In fact, although overexpression of several genes on Chr21, including APP, DYRK1A, SOD1, and RCAN1, lead to impaired neuronal growth and synapse maintenance, at the same time the same genes may also induce an adaption through the action of compensatory pathways during aging. DS may represent an informative model of prodromal AD; thus, promising results may be available by the analysis of DS brain or brain from DS-relevant transgenic mice. Such studies are ongoing in our laboratories.

Abbreviations

DS: Down syndrome
AD: Alzheimer disease
MCI: Mild cognitive impairment
SP: Senile plauques
NFT: Neurofibrillary tangles
Aβ: Amyloid beta peptide
SOD1: Cu-Zn superoxide dismutase
CAT: Catalase
GPX: Glutathione peroxidase
CBS: Cystathionine beta synthase
APP: Amyloid precursor protein
OS: Oxidative stress
Chr21: Chromosome 21
BACE: β-site APP-cleaving enzyme
GATA1: Megakaryocyte-erythroid transcription factor
RCAN1: Regulator of calcineurin1
DYRK1A: Dual-specificity tyrosine(Y)-phosphorylation-regulated kinase 1A.

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