Published in final edited form as:

J Alzheimers Dis. 2011 ; 23(3): 399–409. doi:10.3233/JAD-2010-101335.

Plasma A β as a Function of Age, Level of Intellectual Disability and Presence of Dementia in Down syndrome

Elizabeth Head^{1,2,4,*}, Eric Doran³, Mihaela Nistor¹, MaryAnn Hill^{1,2}, Frederick Schmitt⁴, Richard J. Haier^{1,3}, and Ira T. Lott^{1,2,3}

¹Institute for Memory Impairments and Neurological Disorders, University of California, Irvine, CA, 92697

²Department of Neurology, University of California, Irvine, CA, 92697

³Department of Pediatrics, University of California, Irvine, CA, 92697

⁴Sanders-Brown Center on Aging, University of Kentucky, Lexington, KY, 40356

Abstract

Adults with Down syndrome (DS) are at risk for developing Alzheimer disease (AD). While plasma A β is known to be elevated in DS, its relationship to cognitive functioning is unknown. To assess this relationship, samples from two groups of subjects were used. In the first group, nondemented adults with DS were compared to: 1) a group of young and old individuals without DS and 2) to a group of patients with AD. Compared to these controls, there were significantly higher levels of plasma A β in nondemented adults with DS while AD patients showed lower levels of plasma A β . A larger second group included demented and nondemented adults with DS, in order to test the hypothesis that plasma A β may vary as a function of dementia and ApoE genotype. Plasma A β levels alone did not dissociate DS adults with and without dementia. However, in demented adults with DS, ApoE4 was associated with higher A β 40 but not A β 42. After controlling for level of intellectual disability (mild, moderate, profound) and the presence or absence of dementia, there was an improved prediction of neuropsychological scores by plasma A β . In summary, plasma A β can help predict cognitive function in adults with DS independently of the presence or absence of dementia.

Keywords

apolipoprotein E; neuropsychology; Trisomy 21

Introduction

Middle-aged individuals with Down syndrome (DS) develop Alzheimer disease (AD) pathology [1–4] and as such, are at high risk for the development of dementia [5, 6]. Clinical signs of dementia are more commonly observed when individuals are over 50 years of age [5, 7–9]. By 40 years of age, however, all individuals with DS have neuropathological changes including senile plaques and neurofibrillary tangles (NFT) consistent with AD [2, 3, 10]. Senile plaques contain the β -amyloid peptide (A β) that is derived from a longer precursor protein, β -amyloid precursor protein (APP), the gene for which is on chromosome 21 (21q21.2). DS is a chromosomal disorder caused by triplication of all or part of the 21st

*Corresponding Author: Elizabeth Head, Ph.D. Associate Professor Sanders-Brown Center on Aging University of Kentucky 203 Sanders-Brown Building 800 South Limestone Street Lexington, KY, 40536 Phone: 859-257-1412 Ext 481 Fax: 859-323-2866 Elizabeth.head@uky.edu.

chromosome (trisomy 21). The presence of extra genetic material leads to overexpression of the involved genes and the phenotype of DS is thought to be a consequence of this genetic imbalance. APP gene triplication in DS has been demonstrated to yield excess protein product [11] in peripheral samples (e.g. lymphocytes) and in brain [11, 12]. APP is cleaved by a combination of beta- and gamma-secretase enzyme activity to produce the longer more toxic 42 amino acid long peptide (A β 42) or the shorter, more soluble 40 amino acid peptide (A β 40) [13]. In DS, plasma A β 40 and A β 42 are increased in comparison to those without DS [14–17].

The rationale for studying plasma A β in DS is twofold. First, given the progressive age-dependent nature of AD neuropathology accumulation, we can test hypotheses aimed at early detection of disease by examining adults in middle age prior to the development of clinical signs of dementia. Second, identifying biomarkers of AD for DS may lead to the development of new clinical outcome measures and biomarkers for the testing of therapeutics that may slow or halt AD in DS. The relationship of plasma A β to age is not clear. Although plasma A β concentrations are higher in DS it is not clear whether levels increase with age or actually decrease as peripheral A β is sequestered into plaques, a hypothesis that has been advanced for measures of A β in cerebrospinal fluid from patients with AD in the general population.[18]. Despite life-long overexpression of APP in brain and in peripheral lymphocytes [11, 12], brain A β does not typically begin to deposit until after 30 years [2]. The initial deposits of A β are followed by an acceleration phase that includes NFT and full-blown AD pathology by the age of 40 years [19]. Thus, age-dependent changes in plasma A β may also not be linear if they reflect brain deposition [20].

Consistent with a non-linear age associated accumulation of A β in the brain, it is not surprising that plasma levels of A β in several studies of subjects with DS variably report increases [15, 16], decreases [21] or stable levels of A β with age [22]. Interestingly, Matsubara and colleagues [21] reported that plasma A β is stable until age 50 years and then declines, a finding that is more consistent with significant AD pathology in brain at an age when adults with DS are at the highest risk for developing dementia. However, in a more direct test of differences in plasma A β , the presence or absence of dementia in DS is associated with either no differences [17] or increased A β 42 but not A β 40 [22]. The different outcomes in these two studies may be due to the relatively small sample sizes of demented individuals, the severity of dementia in individual subjects or to other possible differences in the groups of subjects studied. Plasma measures of A β in the general population (non-DS) have also been variable. Increases in plasma A β with age [23] and higher levels in individuals with AD have been reported although there can be overlap with nondemented elderly controls [24]. Longitudinal studies suggest that plasma A β may be elevated in presymptomatic stages of AD but may fall off as dementia develops [25].

In order to explore the relationship of plasma A β levels to age, dementia status, and cognitive functioning, we utilized 2 groups of samples. In the first study, we compared plasma A β levels in a group of nondemented adults with DS to a group of young or old non-DS individuals and to a group of patients with AD to confirm and extend previous reports of increased plasma A β in DS. This first study validated our plasma A β measures and allowed us to test the hypothesis that plasma A β would be reduced with AD and higher overall in DS when compared to controls. In a second, larger group including demented (DS+AD) and nondemented adults with DS (DS) with a wide range of ages, we tested the hypothesis that plasma A β may vary with dementia, with level of intellectual disability, with cognitive performance and with ApoE genotype (not available from our first study).

Materials and Methods

Subjects

Two groups were used in the current study and are summarized in Table 1. The first (Study 1) was described in previous publications [26, 27]. We screened over 40 people with DS between the ages of 34 and 55 identified from a University clinic and from local community care facilities. Individuals with an IQ<45 were not included in the study because subsequent cognitive testing would not be reliable. Seventeen DS subjects were included in this study (WAIS-III FSIQ range 47–70, mean=55.7, SD=6.3). DS subjects received an independent neurological evaluation and none were found to have dementia by DMS-IV criteria. For comparison, we also recruited 17 people with possible or probable AD (MMSE range 9–29, mean =22.6, SD=5.5) from University clinics and community groups; all met DSM-IV criteria for dementia of the Alzheimer's type. Eleven age and sex-matched normal controls for the DS group (MMSE range 27–30, mean=29.2, SD=1.0) and 12 age and sex-matched normal controls for the AD group (MMSE range 27–30, mean=28.5 SD=1.2) were also recruited. These subjects represented normal aging for the non-DS group.

The second group (Study 2) was part of an ongoing longitudinal study of aging in DS. Baseline blood samples were acquired from 52 demented (DS+AD) and 26 nondemented adults with DS (total=78) ranging from 26.5 to 63.2 years of age enrolled in the UCI-ADRC study of aging and a clinical trial. For these subjects, Brief Praxis Test (BPT) [28] scores were available for 61 subjects, 60 subjects had Severe Impairment Battery (SIB) [29] data, and Dementia Questionnaire for Persons with Mental Retardation (DMR)[30, 31] scores were available for 71 subjects. The DMR provides two components reflecting Social (DMRSoc) and Cognitive (DMRCog) function. The level of intellectual disability was established from case file reviews of historical full-scale IQ assessments and subjects were categorized as being mild, moderate or profoundly impaired.

All subjects or their legally-authorized representative gave informed consent in accord with procedures required and approved by the University of California, Irvine Institutional Review Board.

ApoE Genotyping

DNA was isolated from 100ul aliquots of whole blood on an automated nucleic acid purification system (MagNA Pure, Roche Diagnostics). Screening for the three common APOE alleles (E2, E3 and E4) was achieved using PCR-RFLP (polymerase chain reaction - restricted fragment length polymorphism) analysis [32, 33].

Blood collection and plasma (pretreatment)

Plasma samples were collected in EDTA tubes, centrifuged at 4000 rpm, aliquoted and frozen at –80°C until use. A concentrated solution of protease inhibitor cocktail was prepared by dissolving 1 tablet of protease inhibitor cocktail (PIC Complete Mini Roche Cat# 11836153001) in 2 ml PBS. Twenty µl of PIC solution was added to 480 µl plasma. To block cross-reaction of unidentified components in human plasma with mouse IgG in the enzyme-linked immunosorbent assay, the plasma was precleared using methods described previously [23]. Briefly, preclearing was performed by diluting 200 µL of each plasma sample with 350 µL of antigen coating buffer (ACB - 20mM phosphate, 400mM sodium chloride, 2mM EDTA, 0.4% Block Ace; (Dainippon Pharmaceutical, Osaka, Japan), 0.2% bovine serum albumin, 0.0765% 3-with two of these -1-propanesulfonate (CHAPS), pH 7.2), and 50 µL of the Chrompure Mouse IgG, whole molecule – agarose (Jackson Immnoresearch lab, West Grove, PA). After incubation for 2 hours at 4°C, the beads were removed by centrifugation for 1 min at 6000 rpm at 4°C. The plasma was added onto the

plate as is after this preparation (final dilution ~ 1:3). Thus, plasma A β measures reflect “free A β ”.

Sandwich A β ELISA

A β enzyme-linked immunosorbent assays were conducted on the day the plasma was thawed using commercial kits (Human β Amyloid (1-42) ELISA kit Wako Cat# 298-62401 and Human β Amyloid (1-40) ELISA Kit Wako Cat# 298-62301). The 96-well plates were coated with a monoclonal antibody BAN50, against human A β (1–16) as a capture antibody for the N-terminal portion of human A β 1-42. Captured A β from the human plasma sample was detected by a C-terminal specific antibody HRP-conjugated BC05 (Fab' fragment) for A β 1-42 and BA27 for A β 1-40. TMB substrate was added and the absorbance was read at 450 nm on a BioTek Synergy HT micro plate reader.

Data Analysis

Descriptive statistics, statistical analyses, and graphics were executed using SYSTAT (Systat Software, Inc, San Jose, California). A one-way ANOVA was used to compare average plasma A β and the ratio of A β 42:A β 40 for four groups (Young controls – YC, Old controls – OC, probable AD – AD and nondemented DS – DS) of subjects in Group 1. Two pairwise mean comparisons defined *a priori* (with Bonferroni adjustments to probabilities) were carried out for each A β measure. Correlations were computed to compare the relation of the A β measures against age. To compare A β means of DS subjects and controls, two-sample *t*-tests were used. In Study 2, two-way ANOVAS with diagnosis and level of cognitive function as factors and an A β measure or a cognitive score as the dependent variable were used to assess the impact of the factors on the variability of each outcome. Further, two linear multiple regression models were fit to each cognitive/functional score in the analyses of data from Group 2. In the first, dementia diagnosis and level of intellectual disability were predictors. In the second, A β 42 or A β 40 was included as a third predictor. An alpha level less than .05 was used to determine statistical significance.

Results

Within each group of individuals, we first tested for gender differences in plasma A β to determine if this variable should be included as a covariate in subsequent analyses. No significant gender differences were observed ($p>0.05$).

A Comparison of DS to non-DS Controls – Study 1

To confirm that individuals with DS have higher overall levels of plasma A β we compared all 4 groups of subjects (young nondemented DS, young non-DS, AD patients and aged-non-DS controls) in a one-way ANOVA from the first study and found significant differences in plasma A β 42 ($F(3,53)=5.5$ $p=0.002$), A β 40 ($F(3,53)=7.7$ $p<0.0005$) but not for the ratio of A β 42:A β 40 ($F(3,53)=1.73$ $p<0.20$) (Fig. 1A, B, C). Nondemented individuals with DS had 1.66 fold higher levels of plasma A β and adjusted probabilities of tests of pairwise differences in means were significant relative to age-matched controls (for A β 42, $p=0.012$ and for A β 40 $p<0.005$). Further, subjects with AD had lower levels of plasma A β 42 ($p=0.056$) but not A β 40 ($p=0.696$) compared to age matched controls while the A β 42:A β 40 ratio was higher in older controls compared to AD cases ($p=0.028$). Thus, as described in previous reports, we observed significantly higher levels of plasma A β in adults with DS and lower levels of plasma A β in AD patients relative to age-matched controls.

Effects of Age on Plasma A β in DS and Controls – Study 1

We next examined age effects on plasma A β in non-DS cases (n=11 young and n=12 old, total age range between 39–83 years). Figure 1 (D, E) shows that there is a significant correlation between plasma A β 42 and age ($r=0.53$ $p=0.01$) and a weaker correlation with plasma A β 40 ($r=0.43$ $p=0.042$). The correlation with the ratio of A β 42:A β 40 was not significant (Figure 1F). These correlations remained significant when one outlier with very high plasma A β 1-40 was removed. Therefore, both plasma A β 40 and A β 42 appear to increase with age when DS is not present. In contrast, in the DS group, similar age and plasma A β correlation analyses found no significant associations with age.

Effects of Age on Plasma A β in DS – Study 2

Plasma was subsequently collected from a newly recruited second, larger cohort of subjects with DS that included groups of individuals that were either demented or nondemented. This group also included a wider range of ages of adults with DS to extend and replicate the results of the first study with a subset of demented individuals. As seen in Study 1, there were no significant correlations in DS adults between age and plasma A β 40 ($r=-0.015$ $p=0.94$, $n=26$), A β 42 ($r=0.14$ $p=0.51$ $n=26$) and the ratio of A β 42:A β 40 ($r=0.26$ $p=0.19$ $n=26$) (Figure 2A, B, C). A similar lack of association was observed in DS+AD (A β 40 – $r=0.24$ $p=0.09$ $n=52$; A β 42 – $r=0.11$ $p=0.42$ $n=52$; A β 42:A β 40 – $r=-0.12$, $p=0.40$) (Figure 2A, B, C).

Effects of ApoE on Plasma A β in DS in Study 2

We next tested for differences in plasma A β as a function of ApoE genotype, which could not be tested in Study 1 as DNA was not available. ApoE genotype was available for 72 of the 76 subjects in Study 2. Table 2 shows the ApoE genotype of individuals in each of our subject groups as a function of clinical status (DS vs. DS+AD). The proportions of individuals from the entire group falling into E2 (2/2, 2/3), E3 (3/3) and E4 (2/4, 3/4, 4/4) genotypes was 16.6, 33.3 and 50.0% respectively. A univariate ANOVA comparing DS to DS+AD and those with and without an ApoE4 allele on the level of plasma A β shows no differences overall. We also noted that the group of DS+AD had the highest representation of subjects with an ApoE4 allele (20/60, 33.3%) compared to those without dementia (6/33 – 18.2%). Thus, we re-examined the effect of ApoE genotype on individuals with DS+AD alone and found that plasma A β 40 was significantly higher with the presence of ApoE4 ($t(48)=2.21$ $p=0.03$) but not A β 42 ($t(48)<1$ $p=n.s.$). A similar analysis that included only nondemented DS samples did not show ApoE4 associated plasma A β differences.

Dementia, Level of Intellectual Disability, Current Cognitive Function, and Plasma A β in DS – Study 2

In the AD subjects without DS from Study 1, we observed lower plasma A β levels than similarly aged nondemented controls in a cross-sectional comparison. Thus, we hypothesized that DS+AD may have lower plasma A β than DS subjects. As shown in Figure 2, there were no significant differences in plasma A β 40 ($t(76)=-0.58$ $p=0.57$), A β 42 ($t(76)=-1.10$ $p=0.27$) or the ratio of A β 42:A β 40 ($t(76)=0.90$ $p=0.37$) between DS and DS+AD in study 2 (Figure 2, C, D, E). However, individual variability in plasma A β was observed; differences in level of intellectual disability or dementia severity could be related to this variability.

We first addressed the concern that the level of intellectual disability may be related to plasma A β level. A two-way ANOVA including level of intellectual disability (mild, moderate, profound) and dementia diagnosis (nondemented, demented) suggests that average plasma A β 40 and plasma A β 42 do not differ due to level of intellectual disability

(for A β 40, $F(1,70)<1$ $p=n.s.$, A β 42, $F(1,70)<1$ $p=n.s.$, and A β 42:A β 40, $F(1,70)<1$ $p=n.s.$) nor dementia status.

We next assessed the relationship between plasma A β , the level of intellectual disability and presence of dementia on neuropsychological/functional test scores. Figure 3 shows the interaction between level of intellectual disability and the presence of dementia in DS. First, in parallel to the assessment of the relation of level of intellectual disability and diagnosis to plasma A β , a two-way ANOVA was used to assess how these factors contribute to the variability of BPT, the SIB and the DMRSoc and DMRCog subscales. A diagnosis of dementia ($F(1,55)=9.08$ $p=0.004$) and more profound cognitive impairment ($F(2,55)=21.32$ $p<0.0005$) both contribute to lower mean BPT test scores (Figure 3A). Similar outcomes were noted in the SIB with the presence of dementia ($F(1,54)=12.84$ $p=0.001$) and profound level of intellectual disability ($F(1,54)=29.94$ $p<0.0005$) leading to lower mean scores (Figure 3B). On the DMR tests of social and cognitive function, higher scores were associated with presence of dementia (DMRCog – $F(1,65)=50.1$ $p<0.0005$); DMRSoc – $F(1,65)=26.9$ $p<0.0005$) and the level of intellectual disability (DMRCog – $F(2, 65)=32.3$ $p<0.0005$); DMRSoc – $F(2,65)=6.52$ $p=0.003$) (Figure 3C,D).

We computed correlation coefficients (Pearson) between individual test scores and plasma A β 40, A β 42, and A β 42:A β 40 but found no significant associations. However, for the BPT, SIB, and DMRCog scale, more than half their variability is due to a dementia diagnosis and level of intellectual disability. Thus, we used a regression analysis to remove this variability and then to assess the association between plasma A β and the cognitive test scores. The extent of improvement for including plasma A β measures in predicting individual cognitive test scores was assessed using the percentage increase in R^2 due to the addition of the A β measure and also the t statistic for that variable's contribution (Table 3).

Increases in R^2 indicate that plasma A β 42 and A β 40 are associated with BPT and SIB scores after removing the variability of diagnosis and pre-existing impairment from the score. For both DMR scales, including plasma A β 42 levels significantly increased R^2 . Similar effects were observed with adding A β 40 to the model except on DMRSoc. For most regression analyses, one subject with high A β levels was identified as an outlier because its Studentized residual was so extreme. The models were rerun after removing this case and in each regression, the statistical model predictions of cognitive performance were improved. Thus, taking into consideration pre-existing intellectual disability and dementia diagnosis we find that plasma A β can improve the statistical prediction of test performances.

Discussion

We describe the role of level of intellectual disability on plasma A β and how plasma A β (more often A β 1-42 than A β 1-40) may be useful for statistically predicting neuropsychological/functional test scores in adults with DS. Subjects in study 2 were evaluated on several direct tests of function (BPT, SIB) and an informant based measure (DMR). We first established that level of intellectual disability alone did not predict plasma A β . We found that higher plasma A β 1-40 and A β 1-42 was associated with lower BPT scores. However, lower BPT scores could have simply reflected level of intellectual disability as this was a single time point measure. Interestingly, individuals with more severe intellectual disability prior to the emergence of clinical dementia showed a stronger association between BPT scores and plasma A β 1-42 than individuals with milder pre-dementia intellectual disability. However, plasma A β was not associated with scores on either the SIB or on the DMR tests but this may be due to smaller sample size as not all participants had these scores. The association between cognitive function and plasma A β in DS has been described in a few reports but none have observed a significant correlation [14,

34]. This lack of association may be due to the utilization of tests that may not be the most sensitive for dementia or cognitive decline in adults with DS. For example, the MMSE is confounded with low baseline intelligence [35]. Further, MMSE scores also do not predict plasma A β level in studies of sporadic AD [24, 36]. Thus, a novel finding in the current study is that individual variation in the amount of plasma A β in DS may be predicted on the basis of the extent of the existing cognitive impairment but is not due to differences in pre-dementia levels of intellectual disability.

The current study also shows consistent results with previous reports of significantly higher plasma A β 40 and A β 42 in DS relative to age-matched non-DS subjects [14–17, 22]. A more recent study has extended these results to children with DS [37]. In the current study, plasma A β 40 and A β 42 were increased ~1.6 fold in DS as compared to non-DS subjects, which is slightly higher than would be predicted based on a gene dosage effect of APP but is lower than previous publications [14, 17]. However, plasma A β levels and ratios are not linearly associated with age in DS nor are they decreased in DS with AD, which contrasts with our observations in a group of patients with sporadic AD and a previous report in DS [22, 34].

We predicted that age-dependent accumulation of brain A β in DS may be reflected in peripheral measures of A β . But we also predicted, based on previous reports on the age of onset of brain A β that plasma A β changes with age in DS would also not be linear. However, no changes in plasma A β were observed between the ages of 30–40 years when diffuse A β is first visualized in DS brain or from 40–50 years when neuritic A β plaques develop. A drop in plasma A β 42 and an increase in A β 40 was observed in very old DS individuals (>60 years) in a previous study [21]. We and others have also detected significantly lower levels of plasma A β in sporadic AD [36]. However, despite higher levels of plasma A β in DS than non-DS subjects, early signs of AD neuropathology in DS cannot be detected by mean group differences in plasma A β . The lack of age association between plasma A β and the well-established age of onset of AD pathology in the brains of adults with DS [2] also confirms that plasma A β may not reflect brain A β as reported in sporadic AD [38].

A few studies comparing adults with DS with and without dementia using measures of plasma A β [17, 22] have been reported. However, previous studies have included small numbers of subjects with dementia. The current study included 52 adults with (DS+AD) and 24 (DS) without dementia. Overall, the DS+AD were older but when age is included as a covariate to compare differences in plasma A β , no differences were observed. Thus, cross-sectional or single time point measures of plasma A β may not differentiate DS from DS+AD and our results are consistent with a recent larger study [39]. However, it is possible that repeated measures or longitudinal assays can detect changes in plasma A β that may reflect progressive AD neuropathology as suggested in the non-DS population [25]. Further, a longer duration of dementia in DS is associated with higher plasma A β [40].

In the current study, A β 40 was selectively higher in the group of DS+AD but not in DS, which contrasts with some previous studies [16, 37]. However, in another cohort of DS individuals found increased plasma A β 1-42 in subjects who carry an E4 allele [22]. The current study and one by Schupf and colleagues [22] were different in two respects: (1) the current study had fewer subjects without the E4 allele and; (2) a larger number of demented subjects in the current study. The distribution of our subjects into ApoE genotypes was also slightly different from a previous report [41] but this may reflect an enrollment bias towards recruiting DS adults with dementia to the study. This may suggest that ApoE genotype may play a subtle role on plasma A β levels but varies from study to study. Indeed, results from our study that used two separate groups of individuals show significant differences in the amount of plasma A β even when all assays were run together and suggests that multiple

factors may affect outcomes of peripheral assays [42]. For example, it is possible that the time of day the samples were collected or co-morbidities as well as medication history may influence steady-state levels of plasma A β measures [43, 44]. Additionally, recent reports suggest that the duration of dementia in DS might also be an important factor predicting plasma A β [40]. However, to our knowledge, there is limited information regarding the strength of association or correlation between plasma A β measures taken across time in DS and varies between 3–14% in longitudinal studies of non-DS individuals [45].

An additional consideration with A β measures is potential binding to other plasma proteins such as albumin [46] or apolipoprotein J [47]. In addition anti-A β antibodies can be measured in the plasma of DS blood [48] and may also reduce detection of A β . Through pre-clearing our samples, A β attached to these proteins and possibly existing antibodies was most likely reduced leaving free A β that was measured by ELISA. The source of plasma A β may also include larger contributions from peripheral tissues than that reflecting brain (e.g. [49]). However, all plasma samples were treated equivalently during processing suggesting that despite these possible variables contributing to A β level, it still provides useful information with respect to cognition in DS.

In summary, we observe that the amount of plasma A β can improve the prediction of neuropsychological test scores reflecting cognitive function while taking into account the level of pre-existing intellectual disability and presence or absence of dementia. As an extension of previous reports using a larger sample size, we find no differences in a single time point measure of plasma A β between adults with DS with or without dementia. However, in DS with AD, those with an ApoE4 allele show significantly higher plasma A β 40. We are currently following individuals from the second study to determine if changes in clinical measures or in plasma A β levels might predict conversion to AD or worsening of dementia.

Acknowledgments

This project was supported by grant UCI ADRC AG16573 (ITL), HD-37427 (RJH) from NICHD and, in part, by AG21912 (ITL), the “My Brother Joey Neuroscience Fund” (ITL) and DHHS, Eunice Kennedy Shriver National Institute of child Health & Human Development R01 HD064993 (EH, FAS). The authors also thank the subjects and families for participating in the study. Disclosure Statement: The authors have no actual or potential conflicts of interest.

References

- [1]. Mann DMA. The pathological association between Down syndrome and Alzheimer disease. *Mech. Ageing and Develop.* 1988; 43:99–136.
- [2]. Mann DMA, Esiri MM. The pattern of acquisition of plaques and tangles in the brains of patients under 50 years of age with Down's syndrome. *J. Neurol. Sci.* 1989; 89:169–179. [PubMed: 2522541]
- [3]. Wisniewski K, Wisniewski H, Wen G. Occurrence of neuropathological changes and dementia of Alzheimer's disease in Down's syndrome. *Ann. Neurol.* 1985; 17:278–282. [PubMed: 3158266]
- [4]. Hof PR, Bouras C, Perl DP, Sparks DL, Mehta N, Morrison JH. Age-related distribution of neuropathologic changes in the cerebral cortex of patients with Down's syndrome. *Arch. Neurol.* 1995; 52:379–391. [PubMed: 7710374]
- [5]. Lai F, Williams MD. A prospective study of Alzheimer Disease in Down Syndrome. *Archives of Neurology.* 1989; 46:849–853. [PubMed: 2527024]
- [6]. Lott IT, Head E. Down syndrome and Alzheimer's disease: A link between development and aging. *Ment Retard Dev Disabil Res Rev.* 2001; 7:172–178. [PubMed: 11553933]

- [7]. Tyrrell J, Cosgrave M, McCarron M, McPherson J, Calvert J, Kelly A, McLaughlin M, Gill M, Lawlor BA. Dementia in people with Down's syndrome. *Int J Geriatr Psychiatry*. 2001; 16:1168–1174. [PubMed: 11748777]
- [8]. Prasher VP, Filer A. Behavioural disturbance in people with Down's syndrome and dementia. *J Intellect Disabil Res*. 1995; 39:432–436. [PubMed: 8555719]
- [9]. Bush A, Beail N. Risk factors for dementia in people with down syndrome: issues in assessment and diagnosis. *Am J Ment Retard*. 2004; 109:83–97. [PubMed: 15000668]
- [10]. Wisniewski K, Howe J, Williams G, Wisniewski HM. Precocious aging and dementia in patients with Down's syndrome. *Biological Psychiatry*. 1978; 13:619–627. [PubMed: 153156]
- [11]. Rumble B, Retallack R, Hilbich C, Simms G, Multhaup G, Martins R, Hockey A, Montgomery P, Beyreuther K, Masters CL. Amyloid A4 and its precursor in Down's syndrome and Alzheimer's disease. *New Engl. J. Med*. 1989; 320:1446–1462. [PubMed: 2566117]
- [12]. Pallister C, Jung SS, Shaw I, Nalbantoglu J, Gauthier S, Cashman NR. Lymphocyte content of amyloid precursor protein is increased in Down's syndrome and aging. *Neurobiol Aging*. 1997; 18:97–103. [PubMed: 8983037]
- [13]. Selkoe DJ. Normal and abnormal biology of the beta-amyloid precursor protein. *Annu. Rev. Neurosci*. 1994; 17:489–517. [PubMed: 8210185]
- [14]. Cavani S, Tamaoka A, Moretti A, Marinelli L, Angelini G, Di Stefano S, Piombo G, Cazzulo V, Matsuno S, Shoji S, Furiya Y, Zaccaro D, Dagna-Bricarelli F, Tabaton M, Mori H. Plasma levels of amyloid β 40 and 42 are independent from ApoE genotype and mental retardation in Down syndrome. *American Journal of Medical Genetics*. 2000; 95:224–228. [PubMed: 11102927]
- [15]. Mehta PD, Dalton AJ, Mehta SP, Kim KS, Sersen EA, Wisniewski HM. Increased plasma amyloid β protein 1-42 levels in Down syndrome. *Neuroscience Letters*. 1998; 241:13–16. [PubMed: 9502204]
- [16]. Mehta PD, Mehta SP, Fedor B, Patrick BA, Emmerling M, Dalton AJ. Plasma amyloid beta protein 1-42 levels are increased in old Down Syndrome but not in young Down Syndrome. *Neurosci Lett*. 2003; 342:155–158. [PubMed: 12757888]
- [17]. Tokuda T, Fukushima T, Ikeda S, Sekijima Y, Shoji S, Yanagisawa N, Tamaoka A. Plasma levels of amyloid beta proteins Abeta1-40 and Abeta1-42(43) are elevated in Down's syndrome. *Ann Neurol*. 1997; 41:271–273. [PubMed: 9029078]
- [18]. Blennow K. Cerebrospinal fluid protein biomarkers for Alzheimer's disease. *NeuroRx*. 2004; 1:213–225. [PubMed: 15717022]
- [19]. Head E, Lott IT. Down syndrome and beta-amyloid deposition. *Curr Opin Neurol*. 2004; 17:95–100. [PubMed: 15021233]
- [20]. Nistor M, Don M, Parekh M, Sarsoza F, Goodus M, Lopez GE, Kawas C, Leverenz J, Doran E, Lott IT, Hill M, Head E. Alpha- and beta-secretase activity as a function of age and beta-amyloid in Down syndrome and normal brain. *Neurobiol Aging*. 2007; 28:1493–1506. [PubMed: 16904243]
- [21]. Matsubara E, Sekijima Y, Tokuda T, Urakami K, Amari M, Shizuka-Ikeda M, Tomidokoro Y, Ikeda M, Kawarabayashi T, Harigaya Y, Ikeda S, Murakami T, Abe K, Otomo E, Hirai S, Frangione B, Ghiso J, Shoji M. Soluble Abeta homeostasis in AD and DS: impairment of anti-amyloidogenic protection by lipoproteins. *Neurobiol Aging*. 2004; 25:833–841. [PubMed: 15212837]
- [22]. Schupf N, Patel B, Silverman W, Zigman WB, Zhong N, Tycko B, Mehta PD, Mayeux R. Elevated plasma amyloid beta-peptide 1-42 and onset of dementia in adults with Down syndrome. *Neurosci Lett*. 2001; 301:199–203. [PubMed: 11257432]
- [23]. Fukumoto H, Tennis M, Locascio JJ, Hyman BT, Growdon JH, Irizarry MC. Age but not diagnosis is the main predictor of plasma amyloid beta-protein levels. *Arch Neurol*. 2003; 60:958–964. [PubMed: 12873852]
- [24]. Mehta PD, Pirttila T, Mehta SP, Sersen EA, Aisen PS, Wisniewski HM. Plasma and cerebrospinal fluid levels of amyloid beta proteins 1-40 and 1-42 in Alzheimer disease. *Arch Neurol*. 2000; 57:100–105. [PubMed: 10634455]
- [25]. Kawarabayashi T, Shoji M. Plasma biomarkers of Alzheimer's disease. *Curr Opin Psychiatry*. 2008; 21:260–267. [PubMed: 18382225]

- [26]. Haier RJ, Alkire MT, White NS, Uncapher MR, Head E, Lott IT, Cotman CW. Temporal cortex hypermetabolism in Down syndrome prior to the onset of dementia. *Neurology*. 2003; 61:1673–1679. [PubMed: 14694028]
- [27]. Haier RJ, Head K, Head E, Lott IT. Neuroimaging of individuals with Down's syndrome at-risk for dementia: evidence for possible compensatory events. *Neuroimage*. 2008; 39:1324–1332. [PubMed: 18006337]
- [28]. Sano MC, Aisen PS, Dalton AJ, Andrews HF, Tsai W-Y, Consortium IDSAD. Assessment of aging individuals with Down syndrome in clinical trials: Preliminary results of baseline evaluations. *Journal of Policy and Practice in Intellectual Disabilities*. 2005; 2:126–138.
- [29]. Panisset M, Roudier M, Saxton J, Boller F. Severe impairment battery. A neuropsychological test for severely demented patients. *Arch Neurol*. 1994; 51:41–45. [PubMed: 8274108]
- [30]. Evenhuis HM. Evaluation of a screening instrument for dementia in ageing mentally retarded persons. *J Intellect Disabil Res*. 1992; 36:337–347. [PubMed: 1525439]
- [31]. Evenhuis HM. Further evaluation of the Dementia Questionnaire for Persons with Mental Retardation (DMR). *J. Intellect Disabil Res*. 1996; 40:369–373. [PubMed: 8884592]
- [32]. Black SC, Hewett S, Kotubi Y, Brunt RV, Reckless JP. Isoform patterns of apolipoprotein E in diabetes mellitus. *Diabet Med*. 1990; 7:532–539. [PubMed: 2142057]
- [33]. Ossendorf M, Prellwitz W. Rapid and easy apolipoprotein E genotyping using an improved PCR-RFLP technique. *QiagenNews*. 2000; 1:11–12.
- [34]. Matsuoka Y, Andrews HF, Becker AG, Gray AJ, Mehta PD, Sano MC, Dalton AJ, Aisen PS. The relationship of plasma Aβ levels to dementia in aging individuals with down syndrome. *Alzheimer Dis Assoc Disord*. 2009; 23:315–318. [PubMed: 19571732]
- [35]. Lai F, Williams RS. A prospective study of Alzheimer disease in Down syndrome. *Arch Neurol*. 1989; 46:849–853. [PubMed: 2527024]
- [36]. Pesaresi M, Lovati C, Bertora P, Mailland E, Galimberti D, Scarpini E, Quadri P, Forloni G, Mariani C. Plasma levels of beta-amyloid (1–42) in Alzheimer's disease and mild cognitive impairment. *Neurobiol Aging*. 2006; 27:904–905. [PubMed: 16638622]
- [37]. Mehta PD, Capone G, Jewell A, Freedland RL. Increased amyloid beta protein levels in children and adolescents with Down syndrome. *J Neurol Sci*. 2007; 254:22–27. [PubMed: 17275850]
- [38]. Freeman SH, Raju S, Hyman BT, Frosch MP, Irizarry MC. Plasma Aβ does not reflect brain Aβ levels. *J Neuropathol Exp Neurol*. 2007; 66:264–271. [PubMed: 17413317]
- [39]. Jones EL, Hanney M, Francis PT, Ballard CG. Amyloid beta concentrations in older people with Down syndrome and dementia. *Neurosci Lett*. 2009; 451:162–164. [PubMed: 19111900]
- [40]. Prasher VP, Sajith SG, Mehta P, Zigman WB, Schupf N. Plasma beta-amyloid and duration of Alzheimer's disease in adults with Down syndrome. *Int J Geriatr Psychiatry*. 2009
- [41]. Schupf N, Kapell D, Lee JH, Zigman W, Canto B, Tycko B, Mayeux R. Onset of dementia is associated with apolipoprotein E epsilon4 in Down's syndrome. *Ann Neurol*. 1996; 40:799–801. [PubMed: 8957023]
- [42]. Schupf N, Sergievsky GH. Genetic and host factors for dementia in Down's syndrome. *Br J Psychiatry*. 2002; 180:405–410. [PubMed: 11983636]
- [43]. Hoglund K, Wiklund O, Vanderstichele H, Eikenberg O, Vanmechelen E, Blennow K. Plasma levels of beta-amyloid(1–40), beta-amyloid(1–42), and total beta-amyloid remain unaffected in adult patients with hypercholesterolemia after treatment with statins. *Arch Neurol*. 2004; 61:333–337. [PubMed: 15023808]
- [44]. Blasko I, Kemmler G, Krampla W, Jungwirth S, Wichart I, Jellinger K, Tragl KH, Fischer P. Plasma amyloid beta protein 42 in non-demented persons aged 75 years: effects of concomitant medication and medial temporal lobe atrophy. *Neurobiol Aging*. 2005; 26:1135–1143. [PubMed: 15917096]
- [45]. Mayeux R, Tang MX, Jacobs DM, Manly J, Bell K, Merchant C, Small SA, Stern Y, Wisniewski HM, Mehta PD. Plasma amyloid beta-peptide 1–42 and incipient Alzheimer's disease. *Ann Neurol*. 1999; 46:412–416. [PubMed: 10482274]
- [46]. Biere AL, Ostaszewski B, Stimson ER, Hyman BT, Maggio JE, Selkoe DJ. Amyloid beta-peptide is transported on lipoproteins and albumin in human plasma. *J Biol Chem*. 1996; 271:32916–32922. [PubMed: 8955133]

- [47]. Hammad SM, Ranganathan S, Loukinova E, Twal WO, Argraves WS. Interaction of apolipoprotein J-amyloid beta-peptide complex with low density lipoprotein receptor-related protein-2/megalin. A mechanism to prevent pathological accumulation of amyloid beta-peptide. *J Biol Chem.* 1997; 272:18644–18649. [PubMed: 9228033]
- [48]. Conti E, Galimberti G, Piazza F, Raggi ME, Ferrarese C. Increased Soluble APPalpha, Abeta 1-42, and Anti-Abeta 1-42 Antibodies in Plasma From Down Syndrome Patients. *Alzheimer Dis Assoc Disord.* 2009
- [49]. Kuo YM, Kokjohn TA, Watson MD, Woods AS, Cotter RJ, Sue LI, Kalback WM, Emmerling MR, Beach TG, Roher AE. Elevated abeta42 in skeletal muscle of Alzheimer disease patients suggests peripheral alterations of AbetaPP metabolism. *Am J Pathol.* 2000; 156:797–805. [PubMed: 10702395]

Figure 1.

Individual and group differences in plasma A β in Study 1. (A) Plasma A β 1-42 was significantly higher in nondemented adults with DS relative to similarly aged young controls (YC). Patients with AD had lower plasma A β 1-42 than nondemented adults with DS. (B) Plasma A β 1-40 was highest in nondemented adults with DS relative to young controls (YC), old controls (OC) and patients with AD. (C) No differences across groups were noted in the ratio of A β 42:A β 40. Higher amounts of plasma A β 1-42 (D) and A β 1-40 (E) are associated with increased age in individuals without DS. However, the ratio of A β 42:A β 40 was stable across age (F). In the box plots, horizontal lines represent the median, the bottom line is the 25th percentile and the top line is the 75th percentile. Individual data points are shown. Lines indicate significant differences in *post hoc* Bonferroni comparisons at the $p < .05$ level. Lines represent a linear regression.

Figure 2.

Plasma Aβ as a function of age in DS subjects with and without dementia. The correlation between age and plasma Aβ1-42 (A), Aβ1-40 (B) and the ratio of Aβ42:Aβ40 (C) in individuals with DS (with dementia - triangle or without dementia - circle) was not significant. (D) In demented adults with DS, plasma Aβ1-42 was not different from those without dementia. No differences were observed in plasma Aβ1-40 (E) nor the ratio of Aβ42:Aβ40 (F) when comparing demented vs nondemented adults. Lines represent linear regressions. The horizontal line in the middle of each box is the median, the bottom of the box is the 25th percentile and the top is the 75th percentile and individual data points are shown.

Figure 3.

Association between level of intellectual disability, presence of dementia and neuropsychological function. DS adults with dementia scored lower on a direct measure of function in the brief praxis test (A) and in the severe impairment battery (B) with level of intellectual disability also contributing to lower scores. Informant based measures on the DMR cognitive (C) and social (D) subtests revealed higher scores were observed in DS adults with dementia as compared to without dementia. Further, more profound levels of intellectual disability also contributed to higher DMR scores. Bars represent means and error bars, standard errors of the mean.

Table 1

Subject demographics.

	Study 1				Study 2	
	Young Controls	Aged Controls	Probable AD	Nondemented DS	Nondemented DS	Demented DS
Sample Size	11	12	17	17	26	52
Gender (M/F)	5/6	7/5	15/2	9/8	17/9	26/26
Mean Age in years (SE)	46.5 (2.0)	74.2 (1.3)	75.3 (1.8)	44.1 (1.4)	45.1 (1.9)	53.3(0.7)
Range of Ages in years	39–56	66–83	61–91	37–54	26.5–60.2	41.4–63.2
Plasma A β 1–40 Mean in pmol (SE)	45.5 (2.8)	57.7 (4.7)	51.2 (3.0)	75.7 (6.6)	63.7(4.74)	66.9 (3.17)
Plasma A β 1–42 Mean in pmol (SE)	4.4 (0.45)	6.2 (0.6)	4.0 (0.3)	7.3 (1.0)	4.57 (.36)	5.28 (.53)
Plasma Ratio A β 1–42/A β 1–40	0.10 (0.01)	0.11 (0.01)	0.08 (0.01)	0.10 (0.01)	0.09 (0.02)	0.08 (0.01)

Table 2Effect of ApoE genotype and presence of dementia on Plasma A β in DS

Group	N	Demented	At least one E4 allele	A β 1-40	A β 1-42	Ratio A β 1-42/A β 1-40
1	15	-	-	64.72(6.6)	4.57(0.50)	0.098 (0.017)
2	7	-	+	61.39(9.28)	4.14(0.54)	0.0786 (0.024)
3	33	+	-	61.75(4.03)	4.97(0.72)	0.0781 (0.011)
4	17	+	+	76.51(5.07)	5.75(0.79)	0.0754 (0.016)

Table 3

Multiple R² from Regression models

Measure	Base Model	Add Aβ40	Increase	Add Aβ42	Increase
BPT	0.347	0.402	5.5%	0.418	7.1%
SIB	0.471	0.502	3.1%	0.491	2.0%
DMRCog	0.699	0.717	1.8%	0.726	2.7%
DMRSoc	0.356	0.357	0.1%	0.386	3.0%