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The relationship of plasma Aβ levels to dementia in aging individuals with Down syndrome

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Abstract

To study the relationship between plasma levels of amyloid β (Aβ) peptides and dementia in aging individuals with Down syndrome, we investigated the relationship among plasma Aβ, apolipoprotein E genotype and cognitive and clinical factors using baseline specimens form participants in an ongoing clinical trial in individuals with Down syndrome 50 years of age and older. Because of substantial skew in the distribution of peptide levels, analyses utilized log transformations of the data. The ratio of Aβ42 to Aβ40 was associated with the presence of dementia (p=0.003, df=196, F=9.37); this association persisted after adjustment for age, sex level of mental retardation and apolipoprotein E genotype. Consistent with recent reports regarding the effect of presenilin mutations on peptide generation, our finding supports the theory that the ratio of Aβ42 to Aβ40 rather than absolute levels of the peptides is important to the pathophysiology of Alzheimer's disease in genetically susceptible populations.

Keywords

Down syndrome; Aβ; Aβ40/42 ratio; dementia; Alzheimer's disease

Introduction

Down syndrome (DS) carries an extremely high risk of Alzheimer's disease (AD). Autopsy studies show that most individuals with DS have neuropathological evidence of AD pathology by the fourth decade of life. Clinical studies indicate that most develop dementia by the fifth or sixth decade. The leading hypothesis is that this risk results from overexpression of the amyloid precursor protein (APP) leading to increased generation of Aβ peptides, the pivotal molecules in AD pathogenesis. The APP gene resides on chromosome 21, so the trisomy 21 of DS results in 50% overexpression of APP. Of note, an autopsy study of a 78-year old

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individual with Down syndrome caused by a partial trisomy with only two copies of the APP gene found no evidence of AD neuropathology [1].

Measurement of Aβ in plasma has been considered to be a promising biomarker of AD, based on its pivotal role in the disease pathogenesis. Most studies have not shown this measure to be useful in the diagnosis of sporadic AD [2], but levels are markedly elevated in cases of familial autosomal dominant AD, in which there are mutations that increase APP cleavage to release Aβ [3]. In Down syndrome, results to date have been inconsistent [4-7].

We sought to determine the relationship between plasma Aβ and cross-sectional clinical and cognitive aspects of DS during the period of extremely high risk of AD. Using recently reported highly sensitive and specific ELISA methods [8], we have analyzed plasma specimens obtained at baseline from aging individuals with DS enrolled in an international multicenter trial of vitamin E therapy [9].

Methods

Subjects

Aβ levels and genotyping results were obtained using blood specimens collected from the first 198 subjects enrolled in the Multicenter Vitamin E Trial in Aging Individuals with Down Syndrome [9]. This is an ongoing study to determine whether the administration of vitamin E will slow the rate of cognitive/functional decline in older individuals with DS. DS individuals 50 years of age or older are eligible for enrollment; they are recruited from DS clinical programs in the U.S., Canada, the United Kingdom, Ireland and Australia. Subjects must be medically stable and on stable medications (for at least one month. The involvement and cooperation of an informant/caregiver, and an appropriately signed and witnessed consent form are also required. Persons with DS functioning at all levels of mental retardation (MR) are eligible as long as they are able to score above a minimum level on the primary measure described below. The classification of level of MR is based on the DSM-IV [10], utilizing IQ test results when available. The presence of dementia at the time of study enrollment is determined by the site physician using DSM-IV criteria.

Brief Praxis Test

The Brief Praxis Test (BPT) is a modification of the Dyspraxia Scale for Adults with Down Syndrome [11]. The original Dyspraxia Scale was a 62-item scale that had been shown to capture deterioration among persons with DS in the early stage of AD. The BPT consists of 20 items selected from the larger scale because they showed maximum change over a 3-years period among adults with DS [12]. The test can be administered in 30 minutes or less and requires simple behavioral responses with minimal language demands.

Assays

Blood was collected in anticoagulated glass tubes, refrigerated, and shipped overnight with a refrigerant to the central laboratory. Specimens were then separated and aliquoted into polypropylene tubes, and stored at -80° C. Full length Aβ 1-40 and 1-42 was quantified as previously described [8]. For quality control, every plate has an internal reference (human plasma collected from healthy volunteers), the variance in this internal reference is less than ±10%. All peptide level measurements were performed in duplicate; the coefficient of variance of the duplicate measures is 7.9 and 10.8 for Aβ40 and Aβ42, respectively. Consistent with our earlier report on this assay [8], we considered low levels (below 10 fmol/ml) to be below the discriminative range of the assay, and for the purpose of the analyses such levels were considered to be zero.

Because of substantial skew in the distribution of peptide levels, analyses utilized log transformations of the data (zero values were changed to 0.1 to allow log transformation). Assays were conducted in two batches; the results for each batch were standardized separately, combined for analysis and converted to T scores (mean 50, standard deviation of 10) for clarity of reporting. Values are expressed as mean±standard deviation.

Apolipoprotein E genotype was determined by phenotype, i.e., measurement of apolipoprotein E isoforms in plasma, using isoelectric focusing and immunoblotting as previously described [13]. Analysis of the relationship between apolipoprotein E genotype and other data considered genotype as dichotomous (ie, presence or absence of the ε4 allele).

Results

Blood specimens were collected from the first 198 subjects enrolled in the trial for whom Aβ levels and genotyping results were available. Demographic, clinical and cognitive characteristics of the subjects are shown in Table 1. Subjects with dementia were older than those without dementia (56.0 years versus 54.2 years, p=0.004); other characteristics did not differ in these subgroups.

APOE

The prevalence of ε4 allele carriers was 43 of 198, or 22% (including 3 with 4/2 genotype, 37 with 4/3, and 3 with 4/4). Subjects with an ε4 allele were younger (mean age 53.5 versus 55.1, p=0.01). The presence of an ε4 allele was not associated with sex, dementia diagnosis, MR level or BPT score (by analysis of variance with or without adjustment for age).

Plasma Aβ

Aβ assay results are presented in Table 2. A total of 37% of the Aβ42 levels were below the discriminative range of the assay (that is, below 10 fmol/ml); the prevalence of such low levels was not related to the presence of dementia. While raw assays results indicated higher mean levels of Aβ42 than Aβ40, the reverse was true for log transformed data.

Neither Aβ40 nor Aβ42 levels (after log transformation) were related to sex, though the Aβ42/40 ratio was higher in males (1.03 v. 0.96, p=.01, df=197, F=6.67). Peptide levels were unrelated to age. Levels of Aβ40 and Aβ42 were strongly correlated in the full sample (Pearson Correlation =.79, p=0.000), and in the subgroups of non-demented (Pearson Correlation =.81, p=0.000) and demented individuals (Pearson Correlation =.79, p=0.000).

There was an association between MR level and Aβ42 (ANOVA, p=0.028, with two-way comparisons indicating higher levels in profound compared to mild (55.7 v. 49.5, p=0.05), and in severe compared to moderate (51.6 v. 47.9, p=0.05) levels of MR, but not with Aβ40 or the ratio.

Higher values for the ratio of Aβ42/Aβ40 were found in subjects with a diagnosis of dementia compared to those that were not demented (1.05 v. 0.98, p=0.003), though there was no relationship between the presence of dementia and levels of either peptide analyzed separately (Table 3). The ratio of Aβ42/Aβ40 was not correlated to BPT score with or without adjustment for age, sex, MR level and dementia (BPT score was related to MR level [Chi Square p<0.001], but not to presence of dementia). The relationship between the Aβ42/Aβ40 ratio and dementia diagnosis remained significant (p=0.03) in a regression model that included age, sex and MR level (Table 4).

Aβ40 levels were higher in subjects that did not carry the APOE4 allele (50.7 v. 47.3, p=0.05). There was no association between genotype and Aβ42 or the ratio of Aβ42 to Aβ40. When

APOE4 carrier status was added to the regression model, the relationship between the Aβ42/Aβ40 ratio and dementia diagnosis was again significant (p=0.002).

Discussion

The pivotal molecule in the AD brain is the amyloid peptide. In familial autosomal dominant AD, the disease may be caused by increased cleavage of the amyloid precursor protein to release amyloid peptide. In such cases mutations of presenilins (components of the gamma secretase complex) or the amyloid precursor protein augment cleavage; this is reflected in elevated amyloid peptide levels in plasma [3]. In Down syndrome, a likely cause of amyloid accumulation in brain is the extra copy of APP gene which resides on chromosome 21; whether this is reflected in changes in plasma peptide levels remains controversial.

We took advantage of a well characterized group of individuals with Down syndrome at least 50 years of age who enrolled in a clinical trial [9] to study the relationship among amyloid peptide levels and demographic, clinical and genetic data. We used highly sensitive and specific assays for Aβ40 and Aβ42 [8]. In this population, we found associations between peptide levels and sex, APOE genotype, level of mental retardation, cognitive function, and the presence of dementia. The relationship of Aβ42 peptide level to MR level in our study is quite intriguing, suggesting the possibility that amyloid peptides may be involved in the static encephalopathy of DS.

Of particular note, some studies now suggest that it is the ratio of Aβ42 to Aβ40 that causes AD in families carrying presenilin mutations [14, 15]. Presenilin mutations that cause familial autosomal dominant AD are not invariably associated with increased amyloid peptide generation resulting from augmented γ-secretase activity; some mutations modulate γ-secretase activity to increase Aβ42 or decrease Aβ40 without increasing overall levels [15]. Thus, while not all disease-associated mutations increase Aβ production, all do increase the ratio of Aβ42 to Aβ40 [14, 15]. Further, age of onset in familial AD is correlated to both increased Aβ42 and decreased Aβ40, suggesting that the former is toxic while the latter may be protective [14]. It is thus of interest that we found an association between the Aβ42 to Aβ40 ratio and presence of dementia, though levels of the peptides themselves were not related to dementia. We found no relationship between the peptide ratio and BPT score; BPT score was not related to dementia diagnosis, perhaps due to a confounding relationship between BPT score and level of mental retardation.

In sporadic AD, interpretation of plasma Aβ levels has not been straight-forward. Higher plasma levels of Aβ40 [16] or Aβ42 [17] have been reported to show an association with risk of dementia, and with mild cognitive impairment [18-20]. Most studies indicate normal levels of Aβ42 in sporadic AD [2, 18, 21-24], though some investigators find the levels to be high [17] or low [25] in comparison to non-demented controls. Plasma Aβ42 may decline during early AD [17]. One interpretation is that in the presymptomatic and early clinical phases of AD, Aβ42 adheres to plaques, reducing concentrations in the cerebrospinal fluid and plasma.

The literature on plasma Aβ levels in Down syndrome also has not been entirely consistent. Aβ42 levels may be elevated in adults with Down syndrome [4, 6]. One prior study reported no relationship between APOE genotype and plasma peptide levels [5]; however, another study found Aβ42 to be related to APOE genotype [6]. One study has suggested that Aβ42 levels increase with age in Down syndrome [7]; the restricted age range of our study participants (all at least 50 years old) could have obscured such a relationship. Recently reported data from a large Down syndrome cohort suggested a relationship between higher levels of plasma Aβ42 and both risk of dementia and current dementia[26]. In this New York State community-based study of 204 aging individuals with Down syndrome, the risk of incident AD among non-

demented participants was twice as high in those with Aβ42 levels in the highest two tertiles compared with the lowest tertile; further, those with AD at baseline had Aβ42 levels about 13% higher than those who remained dementia-free throughout the multi-year study. Prospective longitudinal studies such as this may be particularly useful in elucidating the role of Aβ peptides in AD pathophysiology. In contrast, our results reflect a cross-sectional evaluation of peptide levels and prevalent dementia.

Differences in assay procedures may have contributed to the variable findings among studies. Published reports have used ELISA assays utilizing antibodies of varying specificity. Multiple secretases cleave APP in the Aβ region, leading to the generation of a number of fragments that may bind to anti-Aβ antibodies. Thus, accurate measurement of full-length Aβ40 and Aβ42 is essential. To quantify these peptides, a sandwich ELISA using specific antibodies against both the N and C terminus end are required. While C terminus end specific antibodies are commonly available, N terminus end specific antibodies are not. We previously developed an N terminus antibody for our Abeta ELISAs [8], our assays specifically detect full-length Aβ40 and Aβ42. Differences in subject characteristics (our trial participants were medically stable, and from several countries) and collection procedures (time of day, fasting status at time of venipuncture, and specimen handling) may also have contributed to different findings in this study compared to the New York State community-based study [26]. For example, our initial collection of blood in glass tubes may have led to reduced Aβ measurements due to adsorption, though analysis of the Aβ42/40 ratio reduces the impact of this issue.

Our findings in Down syndrome support the idea suggested by the presenilin mutation studies that the ratio of Aβ42 to Aβ40 is more salient than absolute level of either peptide as a marker of clinical dementia in genetically susceptible individuals. In the therapeutic trial in which these subjects are enrolled, plasma specimens are again obtained after three years of participation. We will thus have the opportunity to study the relationship among baseline plasma peptide levels, change in levels and incident dementia.

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Abbreviations

Aβ	amyloid β
AD	Alzheimer's disease
APP	amyloid precursor protein
BPT	Brief Praxis Test
DS	Down syndrome
MR	mental retardation

Table 1

Characteristics of the study population at baseline

	Total	**Non-demented (n=148, 25.9%)**	**Demented (n=52, 74.1%)**
Mean age (± standard deviation)	54.7 ± 3.8	54.2 ± 3.6	56.0 ± 3.9
Sex (% male)	61%	60%	64%
Level of mental retardation			
mild	22%	21%	27%
moderate	42%	45%	35%
severe	21%	22%	19%
profound	7%	5%	10%
unknown	9%	7%	10%
Mean score on Brief Praxis Test at screening (± standard deviation)	63.4 ± 13.6	64.6 ± 12.6	60.6 ± 15.6

Table 2

Relationship of peptide levels to presence of dementia.

		Aβ40	Aβ42	T score for log Aβ42	T score for log Aβ40	Ratio T log Aβ42/Aβ40*
Not demented	Mean	288	339	49.6	50.5	.98
	N	145	145	145	145	145
	Std. Deviation	384	577	9.92	9.46	.16
Demented	Mean	242	419	50.9	48.6	1.05
	N	52	52	52	52	52
	Std. Deviation	321	660	10.1	11.4	.25
Total	Mean	276	360	50.0	50.0	1.00
	N	197	197	197	197	197
	Std. Deviation	368	600	9.96	10.0	.19

Mean absolute peptide levels are expressed in fmol/ml. T scores computed separately for two assay batches, then combined for analysis.

*p=0.003, df=196, F=9.37

Table 3

Regression model: ratio of peptide levels (dependent variable is ratio of T scores after log transformation) is significantly associated with presence of dementia after adjusting for age, sex and MR level.

	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error	Beta		
(Constant)	.933	.203		4.586	.000
Age	.003	.004	.065	.891	.374
Sex	-.084	.027	-.228	-3.157	.002
MR level dichotomized	.003	.028	.009	.121	.904
dementia	.065	.030	.157	2.145	.033