Cerebrospinal Fluid Biomarkers and Proximity to Diagnosis in Preclinical Familial Alzheimer's Disease

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Key Words

Cerebrospinal fluid • Biomarkers • Familial Alzheimer's disease, presymptomatic • *PSEN1* gene • *APP* gene • tau protein • β -Amyloid, 42-amino-acid form

Abstract

Background/Aims: Biological markers of utility in tracking Alzheimer's disease (AD) during the presymptomatic prodromal phase are important for prevention studies. Changes in cerebrospinal fluid (CSF) levels of 42-amino-acid β -amyloid ($A\beta_{42}$), total tau protein (t-tau) and phosphorylated tau at residue 181 (p-tau₁₈₁) during this state are incompletely characterized. **Methods:** We measured CSF markers in 13 carriers of familial AD (FAD) mutations that are fully penetrant for causing AD (*PSEN1* and *APP*) and in 5 non-mutation-carrying family members. **Results:** Even among the entirely presymptomatic mutation carriers ($n = 9$), $A\beta_{42}$ was diminished (388.7 vs. 618.4 pg/ml, $p = 0.004$), and t-tau (138.5 vs. 50.5 pg/ml, $p = 0.002$) and p-tau₁₈₁ (71.7 vs. 24.6 pg/ml, $p = 0.003$) were elevated. There was a negative correlation between $A\beta_{42}$ levels and age relative to the family-specific age of dementia diagnosis. **Conclusions:** Our data are consistent with a decline in CSF $A\beta_{42}$ levels occurring at least 20 years prior to clinical dementia in FAD.

Introduction

It is estimated that the prevalence of Alzheimer's disease (AD) will be 11–16 million by 2050 [1]. A better understanding of the biochemical changes occurring during the earliest stages of AD will help us better understand, diagnose, and hopefully develop treatments to prevent it. Decreased cerebrospinal fluid (CSF) levels of the 42-amino acid length derivative of amyloid precursor protein ($A\beta_{42}$) are well characterized in established AD [2]. This is commonly believed to represent a shift in equilibrium from a soluble form in the CSF to its deposition in the central nervous system. Elevated CSF levels of microtubule-associated protein tau (tau) are also found in AD [3] though they are not specific for this disorder [4, 5], suggesting it is a general marker for cerebral damage. Consistent with the hyperphosphorylation of tau observed in the brains of persons with AD, elevated CSF phosphorylated tau (p-tau) is observed in AD and appears to be more specific [5]. This has led to the use of these markers as a commercially available diagnostic test. Decreased CSF $A\beta_{42}$ and increased total tau (t-tau) and tau phosphorylated at residue 181 (p-tau₁₈₁) have also been demonstrated to be predictive of progression [6] and the future development of AD in persons with mild cog-

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nitive impairment [7], providing supporting evidence that the AD pathological process precedes overt dementia by years. At what point during the presymptomatic phase these CSF changes begin to appear is currently incompletely characterized, in part because it is challenging to obtain such samples on normal, middle-aged persons certain to develop AD at a later point.

Unlike late-onset AD in which our ability to predict the illness is imperfect, persons inheriting autosomal dominant mutations causing fully penetrant AD due to mutations in the *PSEN1* and *APP* genes (familial AD or FAD), in whom the illness is essentially fully penetrant, provide the opportunity to study persons in the presymptomatic stage [8]. Moonis et al. [9] described decreased $A\beta_{42}$ and a trend towards increased t-tau and p-tau₁₈₁ in the CSF of 6 presymptomatic FAD mutation carriers. We previously showed that a cohort of 7 FAD mutation carriers (5 presymptomatic) who were an average of 11 years younger than the typical age of AD diagnosis in their family had elevated CSF t-tau and p-tau₁₈₁, and decreased CSF $A\beta_{42}/A\beta_{40}$ ratios compared to non-mutation-carrying family members [10]. Fortea et al. [11] found $A\beta_{42}$ levels to be diminished prior to elevation in tau levels in presymptomatic FAD mutation carriers. In 1 subject studied prospectively by our group who was 22 years prior to the characteristic age of disease diagnosis in his/her family at the initial lumbar puncture and underwent repeat lumbar puncture 4.5 years later, $A\beta_{42}$ levels had diminished and t-tau and p-tau₁₈₁ had increased markedly in the interim [12].

In the current study we evaluated the levels of CSF $A\beta_{42}$, t-tau, and p-tau₁₈₁ in a larger cohort that better enables us to define the levels of such biomarkers in relation to subjects' proximity to the typical age of disease diagnosis in their family.

Methods

Subjects and Clinical Data

Eighteen subjects with or at risk for FAD due to *PSEN1* (A431E, L235V, S212Y) or *APP* (V717I) mutations participated in a comprehensive study assessing clinical, cognitive, imaging, and biochemical changes occurring during the presymptomatic period of FAD. Age of disease onset tends to be consistent within families but varies between them [13], particularly with regard to the A431E *PSEN1* [14] and V717I *APP* [15] mutations represented in this cohort. Therefore, a family-specific 'adjusted age' based on the median age of disease diagnosis in each subject's family was calculated to normalize subjects with regard to their proximity to the time of clinical disease. Evaluations included the Mini Mental State Examination [16] and the Clinical Dementia Rating (CDR) scale [17] performed by an investigator blind to the subjects' mu-

tation status except for 2 subjects who had undergone revealing clinical genetic testing. All subjects signed written informed consent and all procedures were approved by the UCLA Institutional Review Board.

CSF Analyses

CSF was collected at various times of the day within the same week as clinical assessments. CSF was aspirated and placed in polystyrene tubes on ice. Within 2 h CSF was centrifuged and aliquoted into 0.5-ml siliconized polypropylene tubes and frozen at -80°C . CSF was analyzed for $A\beta_{42}$, t-tau, and p-tau₁₈₁ levels using Innogenetics INNO-BIA AlzBio3 multiplex assays on standardized xMAP Luminex technology.

Genetic Analyses

DNA was extracted and apolipoprotein E (APOE) genotyping performed using standard techniques. The presence of the A431E and L235V substitutions in *PSEN1* were assessed using restriction fragment length polymorphism analyses. The patient carrying the S212Y *PSEN1* mutation was ascertained using a commercial test where the open reading frame of the coding region of the *PSEN1* gene was sequenced. The presence of the V717I substitution in *APP* was assessed with direct sequencing. CSF and genetic analyses were performed by personnel blind to all clinical information.

Statistical Analyses

Adjusted age, Mini Mental State Examination scores, and levels of $A\beta_{42}$, t-tau, and p-tau₁₈₁ were compared between mutation carriers (MCs) and noncarriers (NCs) by Student's *t* tests using *p* values for unequal variances. *APOE* genotype frequency was compared between MCs and NCs using χ^2 tests. The Pearson correlation coefficient was used to assess relationships between adjusted age and marker levels. All analyses were performed using PASW Statistics 18.

Results

Thirteen subjects were MCs and 5 were NCs. Eleven MCs had *PSEN1* mutations; 2 had *APP* mutations. Among the 13 MCs, 9 were asymptomatic (CDR scores of 0), 2 were very mildly symptomatic (CDR scores of 0.5) and 1 was mildly (CDR = 1) and 1 was moderately (CDR = 2) demented. There were no significant differences between MCs and NCs in adjusted age, gender, or *APOE* genotype (table 1). Specific data regarding MCs and NCs with respect to gender, absolute age, age range, and mutation for which they were at risk are not shown to maintain confidentiality.

$A\beta_{42}$ levels were significantly lower and t-tau and p-tau₁₈₁ levels significantly higher in FAD MCs relative to NCs (table 1). When only asymptomatic subjects were considered, the differences in $A\beta_{42}$, t-tau and p-tau₁₈₁ between MCs and NCs were diminished but remained significant.

Fig. 1. Levels of CSF biomarkers with age adjusted for family-specific age at disease diagnosis. Lines represent quadratic fit lines with 95% confidence intervals. **a** $A\beta_{42}$ levels drop between 20 and 10 years prior to the expected age of diagnosable dementia. **b** t-tau. **c** p-tau₁₈₁.

Table 1. Descriptive results and results of Student's t tests comparing FAD MCs and NCs

	FAD NCs (n = 5)	All FAD MCs (n = 13)	p values NCs vs. all FAD MCs	Asymptomatic FAD MCs (n = 9)	p values NCs vs. asymptomatic FAD MCs
Adjusted age	-6 ± 14.4	-11.5 ± 10.2	0.47	-16.7 ± 4.6	0.18
MMSE scores	28.8 ± 1.1	25.8 ± 6.4	0.12	28.6 ± 0.9	0.68
APOE genotypes No. 2/3, 3/3, 3/4	0, 3, 2	3, 9, 1	0.17	3, 5, 1	0.23
$A\beta_{42}$, pg/ml	618.4 ± 100.0	354.3 ± 149.3	0.001	388.7 ± 138.4	0.004
t-tau, pg/ml	50.5 ± 9.4	139.0 ± 56.8	<0.001	138.5 ± 59.1	0.002
p-tau ₁₈₁ , pg/ml	24.6 ± 9.2	70.3 ± 31.1	<0.001	71.7 ± 33.6	0.003

Adjusted age is age relative to the median age of dementia diagnosis in the probands' families.

$A\beta_{42}$ levels negatively correlated with adjusted age in MCs ($r = -0.64$, $p = 0.018$). Plotting $A\beta_{42}$ levels against adjusted age (fig. 1a) suggests a substantial decrease occurring between 20 and 10 years prior to the approximate age at which a diagnosis of dementia is anticipated. An increase in t-tau (fig. 1b) and p-tau₁₈₁ (fig. 1c) levels with age in MCs is also suggested though the relation is less strong.

Discussion

In this study of 13 persons carrying FAD mutations, we found diminished CSF levels of $A\beta_{42}$, and increased levels of t-tau and p-tau₁₈₁ relative to non-mutation-car-

rying family members. Consistent with the findings of Fortea et al. [11], these differences persisted when only presymptomatic MCs were analyzed, indicating they occur during the presymptomatic stage. Relating these levels with adjusted age indicates a decline in $A\beta_{42}$ occurring between 20 and 10 years prior to the expected age of disease diagnosis. This is consistent with our serial observation of a subject that showed a decrease in CSF $A\beta_{42}$ between 22 years and 17 years prior to the age of diagnosis in their family [12]. There were trends for t-tau and p-tau₁₈₁ to increase over time in this study and the longitudinal report [12] but the current data suggest more variability in these measures. In our prior structural imaging studies in this same population, we did not find substan-

tial differences between MCs and NCs during the pre- and early symptomatic stage [18], indicating that CSF changes precede measurable cerebral atrophy.

Though change in these CSF biomarkers in relation to pathology is suspected, there is not yet substantial data regarding their utility in predicting the development of AD or of their longitudinal change in presymptomatic persons. A study of elderly nondemented persons found that though baseline CSF A β ₄₂, t-tau, and p-tau₁₈₁ levels were not related to cognitive function, decreasing CSF A β ₄₂ and increasing p-tau₁₈₁ levels over 4 years' time were associated with cognitive decline [19]. Data from the Alzheimer's Disease Neuroimaging Initiative show that decreases in CSF A β ₄₂ were greater in persons with normal cognition than in those with mild cognitive impairment or AD [20], suggesting A β ₄₂ changes can occur prior to symptoms. This study does not address CSF biomarker levels in the presymptomatic state in those certain to develop the disease.

A prior study of CSF biomarkers in persons carrying the *APOE* ϵ 4 allele also demonstrated decreases in A β ₄₂ without changes in tau during the presymptomatic period [21]. Unlike research into the genetic risk of AD conferred by the *APOE* ϵ 4 genotype, our study takes advantage of the predictability of age of disease onset in persons with FAD mutations to estimate the time course of presymptomatic CSF changes. Being a cross-sectional study, we cannot address the intraindividual longitudinal course. Nonetheless, our findings suggest declines in CSF A β ₄₂ occur 20–10 years prior to the expected age of dementia diagnosis though they do not exclude the possibility of earlier changes.

A weakness of our study is that it is not clear to what extent findings in FAD are generalizable to late-onset AD. Though there are many clinical and pathological

similarities between FAD and late-onset AD, FAD can have atypical clinical (e.g. seizures [22], headaches [23]), imaging [24], and pathological [25] features. In addition, age influences CSF biomarker levels and therefore the young age and the relative lack of comorbidity in young FAD mutation carriers makes comparison to late-onset AD more challenging.

As the most commonly cited mechanism by which FAD mutations cause disease is through increasing absolute or relative production of A β ₄₂ and increased plasma A β ₄₂ in these individuals is well described [10, 26], one might expect CSF levels of A β ₄₂ to be elevated prior to its deposition in the brain. Our data do not exclude this possibility; demonstration of this awaits larger FAD data sets such as those being produced by the Dominantly Inherited Alzheimer Network (<http://www.dian-info.org/>) and the Alzheimer's Prevention Initiative. Larger populations such as these are required to disentangle the influences of specific mutation, other genetic (e.g. *APOE*), epigenetic, and environmental influences on the disease process in individuals with FAD. Better understanding of the course of biomarker changes in presymptomatic FAD will help us design proof-of-concept studies for prevention of the illness [27].

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